1 Enteric Human Pathogens Associated with Fresh Produce: Sources, Transport, and Ecology

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Introduction

> Now in the cold parts of the country, don't you think people get to wanting perishable things in the winter—like peas and lettuce and cauliflower? In a big part of the country they don't have those things for months and months. And right here in Salinas valley we can raise them all the year round. ... Do you know we could ship lettuce right to the east coast in the middle of winter?"
>
> John Steinbeck

In 1952, John Steinbeck through his character Adam Trask in "*East of Eden*" commented on the desirability of fresh produce and the uniqueness of the climate and soil conditions of the Salinas Valley of California for providing leafy greens and other vegetables year-round to the rest of the nation. The development of this region on the central coast of California, known as the "Salad Bowl of America," is linked closely to the growth of fresh produce consumption in the U.S. as a result of increased seasonal availability, new varieties of domestic and imported produce, and increased interest in the nutritional and health benefits of fresh produce (Clemens 2004). The growing global economy has continued demand for fresh produce and involves shipping produce long distances rapidly. Increased mechanization and efficiency of production, new and improved cultivars, and new chemicals to treat plant disease and new products have been developed to meet this demand. Minimally processed, bagged produce is a relatively recent new product to help meet the growing demand for fresh produce (USDA-ERS 2001).

An unintended consequence of increased consumption of fresh and bagged produce, however, is an increase in illnesses and outbreaks, including some multistate and multicountry outbreaks. Some of the higher profile outbreaks have been caused by *E. coli* O157:H7–contaminated leafy vegetables, in addition to outbreaks caused by *Salmonella*-contaminated tomatoes, cantaloupe, and other produce items. Investigations of some of these outbreaks have led some to conclude that contamination occurred probably in the field, i.e., preharvest contamination (CalFERT 2007a,b, 2008; Hedberg and others 1999; Gupta and others 2007; Greene and others 2008; Castillo and others 2004).

The leafy green outbreaks appear not to be associated simply with an increase in consumption. Leafy green consumption between 1996 and 2005 increased 9% compared to the previous decade, but outbreaks associated with leafy greens increased 38.6%, with a majority of them caused by *E. coli* O157:H7 (Herman and others 2008).

Outbreaks associated with these commodities occurring since 2000 have led to proposals and active studies to identify the risk factors that may enhance preharvest contamination of produce. However, no single risk factor can explain these multiple outbreaks associated with different production environments, processes, produce items, and pathogens. Rather, it is probable that a convergence of multiple dynamic events involving more than one factor are required to cause major, noticeable outbreaks. Each outbreak may be caused by one or more events different from other outbreaks, even though some common factors are suspected, such as the probable source (e.g., livestock, wild animal) and mechanisms of transport from a source to a field (e.g., watersheds, animal intrusions, aerosols). However, the mechanisms of survival of pathogens in complex environments, and locations and conditions where amplification of their numbers might occur, have not been well documented.

Reviews describing the sources, fate, and transport of pathogens as potential risk factors relevant to preharvest contamination have been published previously; they provide background and specific details that will be summarized in this review. Studies of the incidence and fitness of *E. coli* O157:H7 and *Salmonella* in the produce production environment associated with leafy vegetables, tomatoes, and cantaloupes will be emphasized since they have been associated with multiple outbreaks suspected of being caused by preharvest contamination in the U.S. and Mexico. However, the same environmental factors described for these two pathogens and implicated commodities will apply generally to other pathogens associated with produce contamination, except for specific fitness characteristics that might be linked to a specific commodity. Information related to the incidence and survival of bacterial pathogens and fecal indicators in the production environment, and potential transport processes and risk factors associated with growing fresh produce in dynamic, agricultural regions are presented.

Outbreaks Associated with Selected Fresh Produce Commodities

An unintended consequence of the increased production and consumption of fresh produce is an increase in the number of outbreaks of foodborne illness (CSPI 2007; Sewell and Farber 2001; Sivapalasingam and others 2004). The produce items and types of pathogens associated most frequently with outbreaks in the U.S. (Sivapalasingam and others 2003) and other industrialized countries (Sewell and Farber 2001) have been reported previously, and documented in previous review articles about this subject (Nguyen and Carlin 1994; Beuchat 1996, 2006; Seymour and Appleton 2001; Harris and others 2003; Mandrell and Brandl 2004; Johnston and others 2006b). However, selected data related to outbreaks linked with fresh leafy vegetables and tomatoes will be emphasized in this review in support of the theory that multiple recent outbreaks have resulted from preharvest contamination, especially large multistate or multicountry outbreaks (Table 1.1).

The total number of cases of foodborne illness in the United States has been estimated to be approximately 76 million illnesses per year, associated with 325,000 hospitalizations and 5000 deaths (Mead and others 1999). In a recent review of outbreaks associated specifically with fresh produce, the U.S. Centers for Disease Control and Prevention (CDC) analyzing data from the CDC Foodborne Outbreak Surveillance

Table 1.1. Selected outbreaks associated with enteric human pathogens and fresh produce[a]

Pathogen	Month-Year	Location[b]	No. Ill	Known or Suspected Vehicle	Source Region[c]	Reference
E. coli O157:H7	Jul-95	MT	74	Lettuce, Romaine	MT, WA	Ackers and others 1998
E. coli O157:H7	Sep-95	ME	30	Lettuce, Iceberg	Unknown	CDC 1995
E. coli O157:H7	Sep-95	ID	20	Lettuce, Romaine	Unknown	CSPI 2008
E. coli O157:H7	Oct-95	OH	11	Lettuce	Unknown	CDC 1995
E. coli O157:H7	May-96	IL, CT	61	Lettuce, Mesclun mix	CA (SV)	Hilborn and others 1999
E. coli O157:H7	Jun-96	NY	7	Lettuce, Mesclun	Unknown	CDC 1996
E. coli O157:H7	May-98	CA	2	Lettuce, salad	Unknown	CDC 1998
E. coli O157:H7	Sep-98	MD	4	Lettuce	Unknown	CDC 1998
E. coli O157:H7	Feb-99	NE	65	Lettuce, salad	Unknown	CDC 1999
E. coli O157:H7	Sep-99	CA	8	Lettuce, Romaine	CA (SV)	CDC 1999
E. coli O157:H7	Sep-99	WA	6	Lettuce, Romaine	CA (SV)	CDC 1999
E. coli O157:H7	Oct-99	OH, IN	47	Lettuce, salad	Unknown	CDC 1999
E. coli O157:H7	Oct-99	OR	3	Lettuce, Romaine hearts	CA (SV)	CDC 1999
E. coli O157:H7	Oct-99	PA	41	Lettuce, Romaine	CA (SV)	CDC 1999
E. coli O157:H7	Jul-02	WA	29	Lettuce, Romaine	CA (SV)	CDC 2002
E. coli O157:H7	Nov-02	IL, WI, MN, SD, UT	24	Lettuce	CA (SJoV)	CDC 2002
E. coli O157:H7	Sep-03	CA	57	Lettuce, Iceberg/ Romaine	CA (SV)	CDHS 2004a
E. coli O157:H7	Sep-03	ND	5	Lettuce, mixed with Romaine	Unknown	CDC 2003
E. coli O157:H7	Oct-03	CA	16	Spinach	CA (SV)	CDHS 2004b
E. coli O157:H7	Nov-04	NJ	6	Lettuce	CA (SV)	CDC 2004
E. coli O157:H7	Sep-05	MN	11	Romaine, also vegetables	CA (SV)	MDPH 2006
E. coli O157:H7	Aug/ Sep-05	Sweden	135	Lettuce, iceberg	Sweden	Soderstrom and others 2008
E. coli O157:H7	Aug/ Sep-06	Multi (26 states)	>200	Spinach, baby, bagged	CA (SJuV)	CalFERT 2007b,c
E. coli O157:H7	Nov-06	NJ, NY, PA, DE	71	Lettuce, Iceberg	CA (CentV)	CalFERT 2007a
E. coli O157:H7	Nov/ Dec-06	MN, IA, WI	81	Lettuce, Iceberg	CA (CentV)	CalFERT 2008
E. coli O157:H7	May-08	WA	10	Lettuce, Romaine	CA (SV)	WDOH 2008
S. Saphra	Feb/ May-97	Multi	24	Cantaloupe	Mexico	Mohle-Boetani and others 1999
S. Poona	Spring-00–02[d]	Multi, Canada	58	Cantaloupe	Mexico	MMWR 2002

Table 1.1. *Continued*

Pathogen	Month-Year	Location[b]	No. Ill	Known or Suspected Vehicle	Source Region[c]	Reference
S. Litchfield	Jan/Mar-08	Multi, Canada	51	Cantaloupe	Honduras	CDC 2008a
S. Newport	May/Jun-01	U.K.	19	Vegetables, bagged	Italy, Spain	Sagoo and others 2003
S. Thompson	Oct/Dec-04	Multi, Europe	21	Rucola (arugula)	Italy	Nygard and others 2008
S. Thompson	Mar-99	CA	741	Cilantro	Mexico (suspected)	Campbell and others 2001
S. Javiana	Jun/Aug-90	IL, MI, MN, WI	176	Tomatoes	SC	Hedberg and others 1999
S. Montevideo	Jun/Aug-93	IL, MI, MN, WI	100	Tomatoes	SC	Hedberg and others 1999
S. Baildon	Dec-98–Jan-99	Multi	86	Tomatoes	FL	Cummings and others 2001
S. Javiana	Jun/Jul-02	FL	141	Tomatoes, prediced	?	Srikantiah 2002; Gupta and others 2007
S. Newport	Sep/Oct-02	Multi	510	Tomatoes	VA	Greene and others 2008
S. Braenderup	Jul-04	Multi	125	Tomatoes	FL	Gupta and others 2007
S. Javiana and other serovars	Jul-04	Multi	429	Tomatoes, presliced	?	Gupta and others 2007
S. Newport	Jul/Nov-05	Multi	72	Tomatoes	VA	MMWR 2007a; Greene and others 2008
S. Braenderup	Nov/Dec-05	Multi	82	Tomatoes, prediced	FL	MMWR 2007a
S. Newport	Jul/Nov-06	Multi	115	Tomatoes	?	MMWR 2007a
S. Typhimurium	Sep/Oct-06	Multi	190	Tomatoes	OH	MMWR 2007a
S. Enteritidis	Oct-00–Jul-01	Multi, Canada	168	Almonds, raw	CA	Isaacs and others 2005
S. Enteritidis	Sep-03–Apr-04	Multi, Canada	29	Almonds, raw	CA	MMWR 2004

Table 1.1. *Continued*

Pathogen	Month-Year	Location[b]	No. Ill	Known or Suspected Vehicle	Source Region[c]	Reference
S. Enteritidis	Dec-05–Aug-06	Sweden	15	Almonds, raw	CA	Ledet Muller and others 2007
S. St. Paul	Apr/Jul-08	Multi	>1200	Peppers[e]	Mexico (suspected)	CDC 2008b
Shigella flexneri	May-01	NY	886	Tomatoes	FL	Reller and others 2006
Shigella sonnei	Aug-04	Multi	116	Carrots	CA?	Gaynor and others 2009
Yersinia pseudotuberculosis	Oct-98	Finland	47	Lettuce, iceberg	Finland	Nuorti and others 2004
Yersinia pseudotuberculosis	Aug/Sep-06	Finland	>400	Carrots	Finland	Rimhanen-Finne and others 2009

[a] Outbreaks included have been selected based on location or suspected preharvest contamination. Outbreaks associated with almonds have been included because of recurrent outbreaks suspected of being linked to a common location.

[b] U.S. states are designated by the two-letter abbreviations; Multi = multiple states involved.

[c] SV, Salinas Valley, CA; SJoV, San Joaquin Valley, CA; SJuV, San Juan Valley, CA; CentV, Central Valley, CA. Some location information was provided by California Dept. of Public Health (personal communication). Unknown = traceback not done or incomplete.

[d] Represents three outbreaks (2000, 2001, 2002); the 2000 and 2002 outbreaks were caused by the same strain.

[e] Cases occurred in 43 states, Washington, D.C., and Canada; jalapeño peppers grown in Mexico are suspected as the cause of a majority of cases. Serrano peppers and tomatoes not yet cleared as cause of other illnesses, at the time of preparing this review.

System for 1973–1997, identified 190 outbreaks associated with produce, 16,058 illnesses, 598 hospitalizations and 8 deaths (Sivapalasingam and others 2003). An updated review by CDC of outbreaks associated specifically with leafy greens between 1973 and 2006 determined that 502 outbreaks, >18,000 illnesses, and 15 deaths occurred, with 30 of the outbreaks caused by *E. coli* O157:H7, 35 by *Salmonella,* and 196 by Norovirus (Herman and others 2008). Comparison of the numbers in these two studies reflects the fact that produce-associated outbreaks linked with a known food item increased from 0.7% of all foodborne outbreaks in the 1970s to 6% in the 1990s and has increased further to the present.

The bacterial, viral, and protozoal pathogens associated with fresh produce outbreaks (number of outbreaks) in the U.S. between 1973 and 1997 include the following: *Salmonella* (30 outbreaks), *E. coli* O157:H7 (13), non-O157 *E. coli* (2), *Shigella* (10), *Campylobacter* (4), *Bacillus cereus* (1), *Yersinia enterocolitica* (1), *Staphylococcus aureus* (1), Hepatitis A (12), Norovirus (9), *Cyclospora cayetanensis* (8), *Giardia*

lamblia (5), and *Cryptosporidium parvum* (3); an additional 87 outbreaks were documented without any etiology identified (Sivapalasingam and others 2003). The produce items implicated most frequently in outbreaks are "salad" lettuce, seed sprout, melon and cantaloupe (Sivapalasingam and others 2003).

Multiple sprout outbreaks of *S. enterica* and *E. coli* O157:H7 illness occurring worldwide have been associated usually with sprouts (e.g., alfalfa, mung bean, radish) grown from contaminated seed (Michino and others 1999; Breuer and others 2001; Mahon and others 1997; Proctor and others 2001; Mohle-Boetani and others 2009). The seeds are harvested in different parts of the world (e.g., U.S., Australia, China) under agricultural conditions that in many cases are not controlled well for microbial safety, considering the eventual ready-to-eat product to be produced. The sprouting process involves ideal conditions for enriching even a small concentration of pathogen that may contaminate even a small proportion of the seeds. These conditions emphasize again the importance of the quality of the preharvest environment to produce production at every step of the production cycle, including seed and transplant production, harvesting, and the fields prior to and following harvest (water, fertilizers, crop debris, human and animal visits). Contaminated seeds are not a major risk factor probably in the nonsprout outbreaks to be documented further here; however, seeds should be appreciated as an early preharvest control point in fresh produce production.

Preharvest contamination is suspected in numerous outbreaks associated with leafy vegetables (lettuce and spinach), tomatoes, cantaloupes, and possibly other commodities (e.g., jalapeño peppers, April–July, 2008). For U.S.-grown leafy vegetables alone, there have been more than 20 foodborne outbreaks since 1995 linked to contamination by *E. coli* O157:H7, resulting in at least 600 reported illnesses and 5 deaths. Since 2000, at least 12 outbreaks have been linked to *Salmonella* contaminated tomatoes (>1600 cases) and 3 outbreaks linked to *Salmonella* contaminated cantaloupes (72 cases) (Table 1.1). It is worth noting that, during the final preparation of this review, a major ongoing outbreak of *Salmonella* in St. Paul is associated with jalapeño peppers grown in Mexico and distributed by a company in Texas occurred (CDC 2008b). This was the first reported outbreak associated with this food item; however, additional details will be required to determine whether the contamination occurred on the farm or postharvest (packinghouse). Several outbreaks suspected of being associated with preharvest contamination of tomatoes, lettuce, and carrots by *Shigella* and *Yersinia* species also occurred (Table 1.1). These outbreaks have been listed to emphasize some emerging produce-pathogen issues of concern: preharvest contamination, pathogen persistence and/or fitness in the environment, and diversity of pathogens implicated depending upon local growing conditions (Table 1.1; e.g., leafy vegetables—Western U.S./Sweden/Italy, tomatoes—Eastern U.S., cantaloupe—Mexico, *Yersinia*—Finland).

Previous epidemiological studies of fresh produce outbreaks often lacked definitive evidence of the source of contamination and a step within the food production and processing chain where contamination could have occurred. However, traceback investigations of *E. coli* O157:H7–leafy vegetable outbreaks determined that 12 of them were linked probably to commodity grown on farms in the Salinas Valley, a region located on the Central Coast of California, and the major supplier of fresh produce to the U.S. market (Table 1.1; see references for additional details). Indeed, baby spinach linked to a large multistate outbreak of *E. coli* O157:H7 in the late spring of 2006 was grown in a valley adjacent to the Salinas Valley (CalFERT 2007b;

Cooley and others 2007). Similarly, recurrent outbreaks associated with tomatoes were suspected of being grown on farms in Virginia and Florida, and outbreaks with cantaloupes on farms in Mexico (Table 1.1).

Produce outbreaks linked to a region where a large amount of fresh produce is grown is logical; however, a number of factors revealed by recent outbreak investigations are relevant to concepts of where, when, and how contamination occurs. As noted, outbreaks have been associated with commodities grown in the same region and with preharvest contamination rather than later in the distribution chain (e.g., transport or restaurant). Also, pathogen strains of the same serovar could be isolated from watersheds in the vicinity of implicated fields, and for the first time in recent outbreak investigations, *E. coli* O157:H7 and *Salmonella* strains indistinguishable from the clinical outbreak strains were isolated from environmental samples (CalFERT 2007b, 2007c, 2008; Cooley and others 2007; Greene and others 2008). Therefore, accurate information about the fate and transport processes relevant to contamination processes and the fitness of pathogens near, on, or in produce plants in the field is critical for developing strategies for minimizing preharvest contamination of produce.

Incidence of Human Pathogens on Fresh Produce

How often are produce items contaminated with pathogens? The incidence is very low generally, but any amount may be too much considering the low infectious dose for some of the pathogens, especially *E. coli* O157:H7 on raw produce. The incidence of major foodborne pathogens on different items of fresh produce and in animal hosts has been reported in numerous studies, in addition to data relevant for assessing the survival and fitness of pathogens in agricultural environments such as manure, water, and soil. These data are relevant to consider also for identifying potential point sources and transport processes of pathogens in production environments linked to outbreaks.

Beuchat published in 1996 one of the first and best reviews of reported incidence of common foodborne pathogens on ready-to-eat vegetables, and the potential sources of the pathogens and mechanisms of contamination (Beuchat 1996). The incidence, growth, and survival of foodborne pathogens in fresh and processed produce has been reported also in comprehensive reviews by Nguyen-the and Carlin (Nguyen-the and Carlin 2000) and Harris and others (see Tables I-1 to I-7 in Harris and others 2003), and other recent reviews (Johnston and others 2006b; Beuchat 2006; Mandrell and Brandl 2004). Although distinctions between pre- and postharvest contamination are not provided generally, these reviews provide useful summaries of the different methods for isolating pathogens—for example, *Salmonella*, *Listeria*, *Yersinia*, *Campylobacter* species, *E. coli* O157:H7, and generic *E. coli*—from multiple types of produce items that were grown in different regions of the world.

The incidence of pathogens reported in these separate studies often was between 0 and <10% of all samples tested, with an occasional incidence of >20% reported (Nguyen-the and Carlin 1994; Harris and others 2003; Mandrell and Brandl 2004). Moreover, in the few studies reporting the concentration of pathogen per gram of produce, the levels were low in most studies, even for generic *E. coli,* as a measure of possible fecal contamination. For example, the percentages of positives out of 774 total samples tested for *Salmonella* on leafy vegetables or salad in eight separate studies were 0 (0/151), 0 (0/63), 0.6 (1/159), 0.9 (1/116), 3.5 (2/57), 6.3 (5/80), 7.1

(2/28), and 68% (82/120) (Harris and others 2003). In contrast, all 214 samples of lettuce or salad mix tested for *E. coli* O157:H7 in large U.K. and U.S. studies were negative (Harris and others 2003). Of >3,800 ready-to-eat salad vegetables from retail markets sold in the U.K., only 0.2% were positive for *Salmonella*; an additional 0.5% were considered of poor quality due to contamination with *E. coli* or *L. monocytogenes* at >100 CFU per g of product (Sagoo and others 2003). A survey of "minimally processed" vegetables in Brazil determined that 4 of 181 samples (2.2%) were contaminated with *Salmonella* (Froder and others 2007). Similarly, 180 fresh vegetable samples surveyed in South Africa identified 4 (2.2%) contaminated with *E. coli* O157:H7, and reported levels of *E. coli* O157:H7 as high as 1,600,000 CFU/g of spinach (Abong'o and others 2008). These results reflect the tremendous diversity of produce quality depending upon spatial and temporal factors, and possibly methodological factors.

Multiple outbreaks of *Salmonella* illness associated with tomatoes have occurred recently, but surveys of tomatoes for the incidence of pathogens have been limited. Of 123 samples of domestic (U.S.) tomatoes tested by the U.S. FDA-CFSAN starting in May, 2001, none were positive for *Salmonella* or *E. coli* O157:H7 (FDA-CFSAN 2001b); also, 0/20 imported tomato samples collected starting in March, 1999 were negative for both pathogens (FDA-CFSAN 2001a). However, 11 of 151 imported and 4 of 115 domestic cantaloupe samples in the same surveys were positive for *Salmonella* or *Shigella*. These results appear consistent with the fact that multiple outbreaks occurred in 1997, 2000, 2001, and 2002 due to *Salmonella*-contaminated cantaloupe imported from Mexico (Table 1.1). A large survey of cantaloupe and environmental samples from six farms and packing plants in South Texas and three farms in Mexico resulted in 5/950 and 1/300 cantaloupes positive for *Salmonella*, respectively (Castillo and others 2004). Irrigation-related samples of cantaloupe production (e.g., water source, tank, in field) revealed a higher incidence of *Salmonella* for both Texas and Mexico farms: 13/140 (9.2%) and 10/45 (22.2%), respectively, compared to the commodity. Moreover, generic *E. coli* was isolated at significant levels from some of the samples of Texas and Mexico cantaloupe (3.9%, 25.7%) and Texas and Mexico irrigation water (22.8% and 31.1%, respectively) (Castillo and others 2004). It is noteworthy that none of the 150 field and prewash cantaloupes from Mexico were positive for *E. coli*, compared to 39/75 (52%) and 38/75 (51%) positive samples for the postwash and packed cantaloupe, respectively. Although the concentrations of *Salmonella* and generic *E. coli* in these samples were not reported, these results reflect a prevalence of fecal contamination of water sources (well, river, aquifer, canal, dam), suggesting they may be sources of both pre- and postharvest contamination. Fecal contamination of postharvest processing water is an obvious potential source of cross-contamination of cantaloupes (Castillo and others 2004).

The fitness characteristics of pathogens in the environment are important for their long-term survival and exposure to produce. The long-term persistence in the environment of some foodborne pathogen strains is exemplified by a strain of *S.* Enteritidis implicated in at least one major outbreak, and possibly a minor outbreak, associated with raw almonds in 2000/01 (Isaacs and others 2005) and 2005/06 (Ledet Muller and others 2007), respectively. The *S.* Enteritidis outbreak strain, subtyped as phage type 30, was isolated from a suspect orchard at multiple times over at least a 5-year period,

and with increasing frequency in samples collected during and following harvests (Aug–Dec) and following rain events (Uesugi and others 2007). *Salmonella* strains isolated during the 5-year study were all phage type 30 and indistinguishable from the clinical outbreak strains (or one band difference) by two-enzyme pulsed field gel electrophoresis (PFGE) analysis. Although it was probable that almonds became contaminated by pathogens present in soil/dust where almonds were dropped and then harvested by sweepers, the original source of the outbreak-related strain was never identified, nor were any suspect practices (Uesugi and others 2007).

The extended persistence of any pathogens in an agricultural environment, especially strains that have the potential to cause an outbreak, raises questions relevant to other produce-related outbreaks. Is contamination periodic and cumulative or due to major isolated contamination events? Do persistent strains reflect selection and evolution of special fitness characteristics in a specific environment (e.g., orchard environment; almond, leafy vegetable, tomato surface)? Is the incidence or concentration of pathogens greater now than in the past? Does pathogen survival at low concentrations in harsh soil conditions (dry, high UV) with subsequent resuscitation/amplification (rain/moisture, low UV) relate to virulence? Do certain wildlife species (e.g., mammalian, avian, amphibian) become colonized and high shedders of pathogen and associated with persistent contamination? These and other questions stimulated by recent outbreaks are difficult to answer, but they assist in focusing on areas for further research.

Incidence of Generic *E. coli* on Produce

Increased concerns in the U.S. and other countries about produce-associated outbreaks (Table 1.1) have stimulated initiation of multiple surveys of fresh produce for selected pathogens, and also surveys of the incidence of generic *E. coli* as an indicator of fecal, and potential pathogen, contamination. The results from some of these studies, including recent surveys, are presented to indicate the general microbiological quality of different types of produce grown in different regions conventionally or organically, and tested at different stages of the pre- and postharvest cycle.

A survey of produce items (e.g., arugula, cantaloupe, cilantro, parsley, spinach) collected between November 2000 to May 2002 from 13 farms in the southeastern U.S. revealed *E. coli* levels ranging from 0.7 to 1.5 log CFU/g for field or packing-shed produce (Johnston and others 2005). All samples were negative for *L. monocytogenes* and *E. coli* O157:H7; however, 3 of 398 samples tested for *Salmonella* were positive (0.7%). A similar survey by the same investigators comparing produce grown in the southern U.S. and Mexico involved testing 466 produce items obtained from packing sheds between November 2002 and December 2003. Levels of *E. coli* ranged between 0.7–1.9 and 0.7–4.0 log CFU/g for Mexican and southeastern U.S. produce, respectively (Johnston and others 2006a). All samples were negative for *E. coli* O157:H7, *Salmonella,* and *Shigella*; however, three domestic cabbage samples were positive for *L. monocytogenes* (0.6% of total produce samples; 7% of cabbage samples).

A variety of fresh produce items grown conventionally or organically on farms in Minnesota were picked between May and September 2002 and surveyed for microbiological quality (Mukherjee and others 2004). *E. coli* incidence was 4.3, 11.4, and 1.6%

for 117 certified organic, 359 noncertified organic, and 129 conventional produce items, respectively, and the average *E. coli* counts for the positive samples was reported as 3.1 log MPN/g. The *E. coli* incidence was sixfold higher on organic versus conventional produce and 2.4-fold higher on produce from farms using cattle manure compared to farms using other types of manure. Noncertified organic lettuce had the highest incidence (12/39, 30.8%) for any item with more than 10 samples tested (Mukherjee and others 2004).

The microbiological quality of ready-to-eat produce has been surveyed in other parts of the world. In a study of leafy salads collected from retail markets in Brazil, >85% of 181 samples were reported to have >4 logs *Enterobacteriaceae* per g (Froder and others 2007). Leafy vegetable salads collected postpreparation from 16 university restaurants in Spain yielded 26% positive for *E. coli* (Soriano and others 2001). In contrast, only one (lettuce) of 50 produce items collected from retail and farmers markets in Washington, D.C. was positive for *E. coli* (Thunberg and others 2002). These results suggest major diversity in *E. coli* incidence depending upon the size, time, and location of the study, and possibly differences in the sensitivity of methods.

A study initiated by the USDA Agricultural Marketing Service in 2002 and coordinated with state and other federal agencies to survey the microbial quality of fresh produce items available at terminal markets and wholesale distribution centers continues as of 2008 (USDA-AMS-MDP 2008). The cumulative results over 6 years, with approximately 65,000 samples analyzed to date, provides a significant data set for analyzing spatial, temporal, and other factors related to produce contamination using *E. coli* incidence as the measure of fecal contamination. Multiple commodities, both domestic and imported, have been tested during the program (e.g., cantaloupe, leaf and romaine lettuce, tomatoes, green onions, and alfalfa sprouts) for generic *E. coli*, *E. coli* "with pathogenic potential" (including *E. coli* O157:H7), and *Salmonella*. The results from tests of >59,000 samples from 2002–2007 indicate that low levels of generic *E. coli* are common on produce items collected at the distribution stage of the postharvest production cycle compared to levels on produce in the field (Table 1.2); however, only 1.5 to 2.7% of the samples by year were positive for *E. coli* at concentrations >10 MPN/ml (USDA-AMS-MDP 2008). Moreover, *E. coli* with pathogenic potential based on PCR results for various virulence factors, including shigatoxin 1 and 2 (Stx 1 and 2), ranged from 0.1 to 0.4% of all samples tested each year. Examples of individual produce items having a high percentage of samples positive

Table 1.2. Incidence of *E. coli* on selected fresh produce items obtained and tested in years 2002–2007, as part of the USDA, Agricultural Marketing Service, Microbial Data Program (USDA-AMS-MDP 2008)

Categories	2002	2003	2004[a]	2005	2006	2007[a]
Total no. produce samples tested	10,319	10,972	11,211	11,508	7,646	5,279
No. positive for *E. coli*[b]	759	730	3,226	4,201	1,569	4,420
% positive for *E. coli*	7.4	6.7	28.8[a]	36.5	20.5	83.8
% *E. coli* samples with virulence trait(s)	0.6	0.4	0.4	0.4	0.4	0.1

[a] Generic *E. coli* method was modified in 2004 and again in 2007.

[b] A sample was considered positive if >0.03 MPN/ml rinse solution was determined.

for *E. coli* were cantaloupe (2004 and 2005, 26–32%), leaf and/or romaine lettuce (2004 and 2005, 25–44%), cilantro (2004 and 2005, 66–71%), and parsley (2004 and 2005, 72%) (USDA-AMS-MDP 2008); data not shown.

A similar survey for *E. coli* on 1,183 produce items grown in Ontario, Canada, in 2004 resulted in a 0, 1.3, 6.5, 11.6, 4.9, and 13.4% reported incidence for tomato, cantaloupe, conventional leaf lettuce, organic leaf lettuce, cilantro, and parsley, respectively (Arthur and others 2007a). However, the concentrations of *E. coli* ranged from >5 to 290 CFU/g for leaf lettuce, to <5 to 7,600 and 16,000 CFU/g for cilantro and parsley, respectively. Only two samples yielded a potential pathogen: *S.* Schwarzengrund in a sample each of Roma tomato and organic leaf lettuce (Table 1.2) (Arthur and others 2007a).

Finally, a recent study of 100 domestic bagged cut spinach and lettuce mixes (conventional and organic) for total bacterial, coliform, and *E. coli* counts reported means of 7.0 to 7.7 log CFU/g, <0.5 to >4.0 log MPN/g and 3 to 9.2 MPN/g (16% of samples), respectively, depending upon the product; 12.1% conventional and 16.6% organic spinach and 23.1% conventional and 6.3% organic lettuce mix samples were positive for *E. coli* (Valentin-Bon and others 2008). These results for bagged leafy greens from retail markets are consistent with surveys of ready-to-eat produce in the U.S. and other countries noted above, and other surveys reporting relatively high incidences of *E. coli* in specific produce items such as lettuces, parsley, and cilantro (Soriano and others 2001; Froder and others 2007; USDA-AMS-MDP 2008).

Significant correlations between the levels of *E. coli* contamination of produce and incidences of major bacterial enteric pathogens are lacking. Thus, *E. coli* incidence can be considered simply an indicator of potential minor or major preharvest contamination, and a risk factor for additional postharvest contamination, cross-contamination during washing, or amplification of bacteria (pathogen) during transport and storage. *E. coli* incidence serves as a moderately effective measure of changes in fecal microbial flora during the produce production and processing cycle, and for assessing the potential for pathogenic strains, if they were to be present, to survive under the same produce processing conditions. The concentration of *E. coli* may be a more relevant indicator of the risks associated with human consumption of a contaminated produce item.

Evidence of fecal contamination as high as 50–70% on some produce items does not correlate necessarily to a higher incidence of illness, unless undetected sporadic illness is occurring. Although major outbreaks are of concern, it should be emphasized that relative to the number of consumptions of ready-to-eat produce (and tree nuts) (many billions), outbreaks are not frequent, causing an extremely low number of known total cases per total consumptions; however, some cases are sporadic probably and never linked to a food source. Nevertheless, vigilance and research are important to identify what is probably a rare convergence of events and/or specific circumstances that result in a major outbreak of disease, some of it severe, and thus, a noticeable event. The relatively low incidence of pathogens on produce measured in surveys seems consistent with the speculation that incidence is very rare and occurs only after multiple unusual circumstances that result also in an outbreak. Surveys of produce are informative because they provide a measure of the background incidence of indicators of fecal contamination and pathogens related to dynamic spatial, temporal, and geographic factors. Incidence in the absence of illness or outbreaks also is informative.

Animal Sources of Enteric Foodborne Pathogens Relevant to Produce Contamination

Carriage of pathogens by food animals is a critical factor relevant to many outbreaks associated with produce, meat, milk, and other food products. Evidence for the colonization of cattle (Elder and others 2000; Hussein and Bollinger 2005; Fegan and others 2005; Low and others 2005; Dargatz and others 2003), swine (Chapman and others 1997; Jay and others 2007), sheep (Ogden and others 2005), poultry (Chapman and others 1997; Rose and others 2002; Foley and others 2008; McCrea and others 2006), and multiple species of wild animals (Ejidokun and others 2006; Hernandez and others 2003; Kirk and others 2002; Sargeant and others 1999; Pritchard and others 2001; Wetzel and LeJeune 2006) by *E. coli* O157:H7, *S. enterica*, and *C. jejuni* (Miller and Mandrell 2006) has been documented. Pathogen colonization of livestock and wild animals is a dynamic process depending upon how and when pathogens are encountered in the environment (food, grass, water), pathogen fitness in the environment and animal GI tracts (viability, dose), animal contact/commingling and movement, immunity, and fecal shedding. In addition, there are unknown factors that might enhance or diminish pathogens in particular environments, for example, weather conditions, feed, predation, or antimicrobials. One or more of these factors may be important in initiating or contributing to the size of an outbreak.

Studies documenting the incidence of *E. coli* O157:H7 and *Salmonella* in animals are summarized in Table 1.3. Details regarding the methods, periods, locations, and samples studied can be obtained from the original papers cited.

E. coli *O157:H7 and Non-O157 STEC*

Cattle are major carriers of *E. coli* O157, non-O157 shigatoxin-positive *E. coli* (STEC), *S. enterica* and *C. jejuni* strains (Table 1.3). Strains of the same serovars as those associated with produce outbreaks have been isolated frequently from cattle. Similarly, sheep, pigs, chickens, and turkeys are common or intermittent carriers of these pathogens, and a variety of wildlife species carry these pathogens or related pathogens (Tables 1.1 and 1.3). For example, *E. coli* O157:H7 and non-O157 STEC strains have been isolated from deer (Keene and others 1997; Sargeant and others 1999; Fischer and others 2001; Dunn and others 2004; Renter and others 2006), feral swine (Jay and others 2007), pigeons (Morabito and others 2001), seagulls (Makino and others 2000), starlings, horses, dogs (Hancock and others 1998), barn flies (Keen and others 2006), and slugs (Sproston and others 2006). *Salmonella* has been isolated from deer (Branham and others 2005; Renter and others 2006), badgers (Nielsen and others 1981), wild mice (Tablante and Lane 1989), wild turtles and tortoises (Hidalgo-Vila and others 2007), and a variety of wild birds (Fenlon 1981; Wahlstrom and others 2003; Hughes and others 2008). The concentration of pathogen in wildlife samples is not well documented; thus, the shedding status of wildlife compared to livestock is unclear. Moreover, the quantity of feces shed by different species of wildlife per animal or for a population in a region is unknown, so data relevant to the total amount of pathogen disseminated by a species in any spatial and temporal context also are unknown. The amount of pathogen shed by an animal is extremely relevant epidemiologically for identifying

Table 1.3. Selected studies reporting incidence of *E. coli* O157, *S. enterica,* and *C. jejuni* in livestock and wild animal feces

Pathogen	Animals	Incidence in Feces	Reference
E. coli O157	Beef cattle U.S. (multiple states) and multiple countries	0.3–19.7%, feedlot[a] 0.7–27.3%, pasture 0.9–6.9%, range 0.2–27.8%, slaughter	Review of 39 separate studies: Hussein and Bollinger 2005
E. coli O157	Beef cattle (Scotland)	3.4%, some high shedders[b]	Matthews and others 2006
E. coli O157:H7	Beef cattle Dairy cattle U.S. (multiple states)	3.6% 3.4%	Doane and others 2007
E. coli O157:H7	Beef cattle, hides U.S. (multiple states)	9–85%	Arthur and others 2007b
E. coli O157:H7	Zebu ("humped cattle")	5.4%	Tuyet and others 2006
Non-O157 *E. coli*	Beef cattle U.S. (multiple states) and multiple countries	2.1–70.1% overall 4.6–55.9%, feedlot 4.7–44.8%, grazing 2.1–70.1%, slaughter	Review of 21 separate studies: Hussein and Bollinger 2005
E. coli O157	Sheep (U.K.)	6.5%, some high shedders[b]	Ogden and others 2005
E. coli O157	Sheep (U.K.)	2.2%	Chapman and others 1997
E. coli O157	Sheep (U.K.)	0.7%	Milnes and others 2008
E. coli O157:H7	Sheep (U.S.)	4.4%	Keen and others 2006
E. coli O157:H7	Sheep (Spain)	7.3%	Oporto and others 2008
E. coli O157	Pigs (U.K.)	0.4%	Chapman and others 1997
E. coli O157:H7	Pigs (U.S.)	2.0%	Feder and others 2003
E. coli O157:H7	Pigs (Japan)	1.4%	Nakazawa and Akiba 1999
E. coli O157	Pigs (U.K.)	6.7%	Cooper and others 2007
E. coli O157	Pigs (U.K.)	0.3%	Milnes and others 2007
E. coli O157:H7	Pigs (U.S.)	1.2%	Keen and others 2006
E. coli O157:H7	Pigs (U.S.)	8.9%	Doane and others 2007
E. coli O157:H7	Feral swine (U.S.)	14.9%[c]	Jay and others 2007
E. coli O157:H7	Chickens Turkeys (U.S.)	0.9% 7.5%	Doane and others 2007
E. coli O157	Chickens (U.K.)	3.8%	Cooper and others 2007
E. coli O157	Goats (U.K.)	28%	Cooper and others 2007
E. coli O157:H7	Deer (US) 3/32 pellets	9.4%	Keene and others 1997
E. coli O157	Deer (U.S.)	2.4%[d]	Sargeant and others 1999
E. coli O157:H7	Deer (U.S.)	0.5%[e]	Fischer and others 2001
E. coli O157:H7	Deer (U.S.)	0.25%[f]	Renter and others 2001
E. coli O157:H7	Deer (U.S.)	0.3–0.4%[g]	Dunn and others 2004
E. coli O157	Rabbits	?	Pritchard and others 2001; Leclercq and Mahillon 2003
E. coli O157:H7	Ducks	?	Leclercq and Mahillon 2003

Table 1.3. *Continued*

Pathogen	Animals	Incidence in Feces	Reference
E. coli O157:H7	Fish	4.7%	Tuyet and others 2006
E. coli O157	Rats (Norway)	40%[h]	Cizek and others 1999
Non-O157 EHEC	Rabbits	9–25%[i]	Garcia and Fox 2003
S. enterica	Cattle (U.S.)	6.3%	Dargatz and others 2003
S. enterica	Cattle (U.S.)	4.4%	Barkocy-Gallagher and others 2003
S. enterica	Cattle (Australia)	4.5%, grass-fed 9.0%, feedlot	Fegan and others 2004
S. enterica	Cattle (U.K.)	1.4%	Milnes and others 2007
S. enterica	Cattle (U.S.)	13–72%, cows 20–71%, calves	Pangloli and others 2008
S. enterica	Dairy (U.S.)	60–63%, soil 53–67%, water 46–71%, air 13–63%, bird feces 24–85%, insects Feed, 21–92%	Pangloli and others 2008
S. enterica	Goats (U.S.)	3.7%	Branham and others 2005
S. enterica	Sheep (U.S.)	7.3%	Branham and others 2005
S. enterica	Sheep (U.K.)	1.1%	Milnes and others 2007
S. enterica	Pigs (U.K.)	23.4%	Milnes and others 2007
S. enterica	Poultry (U.S.)	50.8%, transport pads 18.7%, flies 14.2%, drag swabs 12%, boot swabs	Bailey and others 2001
S. enterica	Poultry (U.S.)	10.5%, by flocks 1.1%, by row	Kinde and others 2004
S. enterica	Poultry	13.0%, by flocks	Rasschaert and others 2007
S. enterica	Deer (U.S.)	7.7%, rumen	Branham and others 2005
S. enterica	Deer (U.S.)	1.0%	Renter and others 2006
S. enterica	Wild tortoises Wild turtles (Spain)	100% 12–15%	Hidalgo-Vila and others 2007
S. enterica	Wild birds (U.S.)	1.2–3.2%	Kirk and others 2002
S. enterica	Wild birds (U.K.)	0.015%	Hughes and others 2008
Salmonella	Seagulls	12.9%	Fenlon 1981
C. jejuni/C. coli	Cattle, chickens (live), geese, ducks, pigs, sheep (Multiple countries)	0–100%[j]	Review of >20 studies; Miller and Mandrell 2006

[a] Ranges of incidence reported for multiple studies; majority of isolates were *E. coli* O157:H7.
[b] High shedders, >10,000 CFU/g; majority positive for Stx2.
[c] Collected from one ranch in California.
[d] 5/212 white-tailed deer.
[e] 3/609 individually sampled deer, 1997 and 1998.
[f] 4/1608 mostly white-tailed deer, Nebraska, 1998.
[g] 1 of 338 hunter-harvested deer, 1 of 226 captive herd deer, Louisiana, 2000–01.
[h] 4 of 10 rats; however, negative for H7.
[i] Laboratory rabbits; all EHECs positive for Stx1.
[j] Cattle, 62% average for 14 studies; chicken (live), 64% for 20 studies; geese/ducks, 55% for 6 studies.

potential sources of pathogen and relevant risk factors for contamination of produce (Chase-Topping and others 2007).

The incidence data listed in Table 1.3 are from selected recent studies; the data reflect the dynamic nature of the incidence associated with different animal hosts, spatial and temporal differences, and a variety of different methods. In a recent review by Hussein and Bollinger, 39 reported studies of the incidence of *E. coli* O157:H7 in thousands of cattle fecal samples from feedlots, pasture/range, and entering slaughter ranged from 0.2 to 28%, depending upon the study and the cattle feeding or production process (Hussein and Bollinger 2005). A previous review of some of the same studies involving animals in Asia, Australia, Europe, and North America (sampling periods between 1991–1999) reported incidence in fecal samples in the range of 0.1 to 62% (Duffy 2003). Indeed, the common occurrence of *E. coli* O157:H7 in cattle is consistent with numerous outbreaks of *E. coli* O157:H7 occurring as a result of direct human contact with animals, feces, or manures at fairs, farms, and other public settings (Duffy 2003; Durso and others 2005; Keen and others 2006, 2007). Similar studies of sheep in the U.K., U.S., and Spain, representing thousands of samples, reported an incidence of *E. coli* O157 that ranged from 0.7 to 7.3%, and for domestic pigs incidence ranged from 0.3 to 8.9% (Table 1.3).

In multiple studies of cattle feedlots and ranches, strains of *E. coli* O157:H7 persisted for up to 24 months at individual farms, and strains indistinguishable by molecular typing methods were isolated from farms separated by up to 50 km (Rice and others 1999; LeJeune and others 2004; Wetzel and LeJeune 2006). Indeed, a link between livestock and human illness with *E. coli* O157:H7 and other STEC has been supported by a direct correlation reported between the density of livestock and amount of reported illness in a region of Ontario, Canada (Michel and others 1999).

Salmonella enterica

Strains of *S. enterica* were isolated from 1.4 to 9% of beef cow fecal samples (Australia, U.S., U.K.) reported in four studies (Table 1.3). In a recent study of 7,680 animal and environmental samples from a single U.S. dairy, 13–72% of the cattle samples (depending upon period of testing), and >50% of air, soil, water, insect, and bird feces samples yielded *S. enterica* (Pangloli and others 2008). Similarly, high incidences of *S. enterica* in pigs were reported in a U.K. study (23.4%), in poultry flocks (10.5 to 13%) in U.S. and Belgium studies, and in poultry production environmental samples (12 to 51%) in a U.S. study (Table 1.3). *S. enterica* has been isolated from 1 to 7% of deer samples in two studies reported and up to 3% of wild bird samples. A multidrug-resistant *S.* Newport strain was prevalent on two different farms for months and shed by a cow for at least 190 days (Cobbold and others 2006), and, as noted above, a strain of SE (PT30) has been isolated from almond orchard soil periodically for at least 5 years (Uesugi and others 2007).

Campylobacter *Species*

C. jejuni incidence in cattle, poultry, other farm animals, and wild animals has been reported and reviewed (Miller and Mandrell 2006). Although the incidence of *C. jejuni* reported in >20 studies is comparable or higher than those reported and listed for *E. coli* O157 and *Salmonella* in Table 1.3, few major outbreaks *of C. jejuni* associated

with fresh produce have occurred (Mandrell and Brandl 2004). In agreement perhaps, is the absence of any isolation/detection of *C. jejuni* on >6,800 produce samples in recent studies reported (Sagoo and others 2001; Thunberg and others 2002; Moore and others 2002; Sagoo and others 2003), suggesting that *C. jejuni* may be of lesser fitness compared to *E. coli* O157 and *Salmonella* in environments relevant to fresh produce production and preharvest contamination (Brandl and others 2004). Nevertheless, high numbers of sporadic *C. jejuni* illnesses compared to *E. coli* O157 and *Salmonella* (MMWR 2005b, 2007b) suggest surveillance to identify food sources associated with *C. jejuni* illness, including produce, should be continued.

The results summarized in Table 1.3 confirm there are multiple livestock and wildlife sources of pathogens and suggest modes of transport of pathogens for contamination of fresh produce in fields or orchards. Livestock are located near produce production in many locations, but not close enough usually to be considered a major risk. However, resident wildlife species are potential sources of pathogens also, and commingle with livestock on ranches, dairies, or feedlots, thus increasing exposure of livestock and wildlife to pathogens. Wildlife colonized by pathogens will roam and potentially disseminate them to produce or other locations in the vicinity of produce (Jay and others 2007). This presents problems for controlling wildlife intrusion into fields depending upon the size and roaming capability of the species. Small mammals (e.g., squirrels, mice, raccoons), large mammals (feral swine, deer, elk), and birds illustrate the diversity of population sizes, barriers (fencing height, depth, gage), and habitat that are issues in considering interventions to control exposure of wildlife to fields. Therefore, only obvious risk factors can be addressed until definitive data are obtained about major sources of pathogen in an environment.

A few conclusions can be drawn from the selected livestock and wildlife incidence data. First, they reflect the dynamic fluctuations in the incidence of enteric pathogens that can occur and that relatively high incidence of certain pathogens may occur at specific times. Second, there appears to be a general trend in higher incidence of *S. enterica* strains in surveys of animal and environmental samples compared to *E. coli* O157:H7, a trend consistent with the general amount of illness reported for these pathogens in the U.S. and U.K. (MMWR 2005b, 2007b; CDR 2006). In contrast, the recurrent outbreaks of *E. coli* O157:H7, in the absence of any known *Salmonella* outbreaks, associated with leafy vegetables grown in the same region (Table 1.1) is inconsistent with this trend. Perhaps, a study of the incidence of *Salmonella* in the environment of leafy vegetable production would provide clues to explain this paradox.

High-level Shedding of *E. coli* O157:H7 and *Salmonella* by Some Animals

Measuring the prevalence of pathogens in animals and other environmental reservoirs relevant to produce production are informative, but the concentration and total amount of pathogen disseminated is perhaps more relevant to identifying potential risks in a produce production region. However, quantifying pathogen in complex samples is difficult due to the inability to survey livestock and wildlife populations comprehensively and to obtain accurate values with environmental samples containing low concentrations of pathogens in a complex microbial flora.

Cattle shedding high levels of *E. coli* O157:H7 in their feces have been identified in some surveys. The majority of cows positive for *E. coli* O157:H7 in a herd have <100 CFU/g of feces, and this usually is detectable only by preenrichment and immunomagnetic selection methods. However, high-level shedders ("super shedders") have been identified that shed between 1,000 and 1,000,000 CFU/g of feces (Low and others 2005; Chase-Topping and others 2007). Similarly, mice shedding $>10^8$ CFU viable *Salmonella* cells per gram of feces have been identified in laboratory studies, and high-shedding status appeared linked directly to the health of the intestinal microflora and level of inflammation in the colon (Lawley and others 2008).

Indeed, models of prevalence, heterogenous shedding, and human infectious dose data are consistent with the "80/20 rule" suggesting that 80% of the transmission of an infectious agent results from the 20% of the most infectious members of the population (Matthews and others 2006). Therefore, colonized animals shedding large doses of a pathogenic strain (or strains) relative to the majority of a herd, or any population, in a region are relevant epidemiologically because the strains they shed are likely to be predominant in the environment. If predominant strains are virulent members of the species also, they are candidates for outbreaks of foodborne illness or other forms of infectious disease (Matthews and others 2006).

Other factors important epidemiologically are the survival of a virulent pathogen in complex environments and its fitness in water, in soil, and on field crops. It is noteworthy then that *E. coli* O157:H7 strains linked to four outbreaks associated with bagged leafy vegetables in 2005 and 2006 (including the baby spinach outbreak, 2006) appear to be part of a phylogenetically distinct group ("clade 8") that includes virulent strains associated with outbreaks from patients who had been hospitalized with hemolytic uremic syndrome and strains associated with increased frequency of hospitalization (Manning and others 2008).

Increased virulence correlates also with a lower infectious dose required for illness. The estimates of the dose of *E. coli* O157:H7, for example, capable of causing illness in a population exposed to contaminated food ranges from 4 to <40 CFU/g of food (Strachan and others 2001; Teunis and others 2004). Thus, a more virulent strain capable of causing illness at an even lower infectious dose emphasizes the risks associated with any pathogen contamination of environments near produce production.

Incidence of Potential Pathogens in Municipal and Agricultural Watersheds

Pathogens shed onto soil on the range, in feedlots, or in other habitats are dispersed and disseminated further by runoff into watersheds. Table 1.4 summarizes the results of some selected recent studies of the incidence and fitness of *E. coli* O157:H7 and *S. enterica* in municipal or agricultural watersheds because they have been the bacterial pathogens linked most frequently with recent outbreaks associated with preharvest contamination of fresh produce (Table 1.1).

The incidence of *E. coli* O157:H7 in watersheds has been reported to be low generally (<2%) compared to *Salmonella*, reflecting probably the general concentration of the pathogens in the water samples. Strains of *E. coli*, potentially pathogenic based on the presence of known virulence genes (*tir* and *stx*), were isolated frequently in one U.S. study, indicating that specific urban watersheds can be contaminated heavily

Table 1.4. Incidence of pathogenic *E. coli* and *S. enterica* in municipal and agricultural watersheds

Description	Pathogen	Reference
Alberta, Canada; watershed near agriculture; 1999–2000; not associated with manure output	*E. coli* O157:H7 (13/1483 = 0.9%) *Salmonella* (88/1429 = 6.2%)	Johnson and others 2003
Baltimore, MD, area, U.S.; 2002–04; potential pathogens	*E. coli*, *tir* and/or *Stx-pos* (653/1218 = 53%)	Higgins and others 2005
California, central coast, U.S.; agricultural and urban watershed; 2005–06	*E. coli* O157:H7 (38/584 = 6.5%)	Cooley and others 2007
France, near Mediterranean; agricultural and urban; 1996–97	*Salmonella* (574/? = ?)[a]	Baudart and others 2000
Central African Republic; N'Goila	*E. coli* O157:H7 (6/260 = 2.3%)	Tuyet and others 2006
Cornwall, U.K.; freshwater stream crossing beach; 2004; outbreak-associated	*E. coli* O157:H7 (5/? = ?%)[a]	Ihekweazu and others 2006
Georgia, U.S.; single day, 83 sites on river; 2005	*Salmonella* (62/83 = 75%)	Meinersmann and others 2008

[a]Total number of samples tested was not noted.

with potential pathogens (Higgins and others 2005). However, the lack of any evidence of human illness associated with these strains suggests that they are not highly virulent compared to *E. coli* O157:H7.

Four of the studies listed in Table 1.4 were initiated as a result of high levels of illness and outbreaks of *E. coli* O157:H7 associated with exposure to water or food (Johnson and others 2003; Ihekweazu and others 2006; Tuyet and others 2006; Cooley and others 2007). One of these studies of a watershed in a major leafy vegetable production region of the U.S. was initiated as a result of three separate outbreaks of *E. coli* O157:H7 linked to leafy vegetables grown in the Salinas Valley region of California (Table 1.1), and possibly linked to a single farm (Cooley and others 2007). Water samples obtained monthly on average from <20 sites, most within approximately 30 km of one another, revealed that >6% of the samples were positive for *E. coli* O157:H7. Sites nearby cattle grazing in elevated regions of the watershed were positive more frequently, and samples obtained during or subsequent to heavy rain events with increased water flow correlated with increased incidence at specific sites. Also, strains indistinguishable or highly related by genotype were isolated at the same time up to 30 km apart, or from the same sites months apart (Cooley and others 2007). Similarly, outbreak investigations of farms and ranches in the central coast region of California have provided clues to intriguing fate and transport relationships from assessment of genotypes of strains of *E. coli* O157:H7 isolated from environmental and wildlife samples obtained at similar times and locations (Cooley and others 2007; Jay and others 2007). Predominant strains may be persistent in some environments and transported by the commingling of wildlife and livestock into watersheds and possibly fields where produce is grown. However, the amounts of pathogen, their

persistence in soil, and the processes involved in exposure of seedlings or mature plants to pathogen are unclear. In the absence of an effective "kill step" for postharvest produce, it remains important to identify sources of pathogens and their fate and transport in produce environments; this may assist in development of strategies for preventing contamination of produce destined for the ready-to-eat market.

Fate and Transport of Human Pathogens in the Environment

It has been difficult to determine the primary source of preharvest produce contamination; however, nearby livestock, poultry, or other farm animals are obvious potential point sources for further dissemination in the environment, and linked possibly to produce (Table 1.3). Potential mechanisms for dissemination of pathogens from contained farms or feedlots are movement of livestock to new locations, wildlife intrusion, water runoff/flooding (Table 1.4), dust/bioaerosols, manure/compost/compost-tea fertilizers, and possibly other intra- and interfarm human activities (farm vehicles and equipment).

For pathogens to be transported outside an animal host, they must remain fit enough to survive (and possibly grow) until they encounter an environment favorable for growth. Findings from previous studies measuring the survival of pathogenic *E. coli* and *Salmonella* in manure, soil, and water are relevant to hypotheses about how preharvest contamination occurs. Table 1.5 is a list of selected studies that provide a comparison of measured fitness characteristics of *E. coli* O157, *E. coli* O157:H7, and *Salmonella* in environments relevant to fresh produce contamination, including manure, soil, manure-amended soil, and water. It is worth noting that some of these studies report the incidence of pathogens in their natural state in relevant environmental samples, whereas others involved spiking samples with marked strains and then monitoring their incidence over a period of time.

Each study listed in Table 1.5 involved different locations and experimental conditions; however, it is noteworthy that outcomes generally were consistent. For example, in nearly all studies, *E. coli* O157 or *E. coli* O157:H7 remains detectable in some samples for >30 days, but longer than 6 months in other samples (Table 1.5; cow water trough, sheep manure, manure-amended soil). *Salmonella* cells were detectable for similar periods of time (e.g., soil, manure-amended soil), but an outbreak strain was detectable for >1500 days in soil samples from an almond orchard linked to the outbreak (see below). Similarly, multiple strains of *E. coli* O157 were isolated for months from biofilms on flint shingles immersed in stream beds exposed to runoff from farm animals positive for the pathogen (Cooper and others 2007).

These studies support the persistence theory and possible mechanisms of periodic reintroduction of pathogens in agricultural environments. Conversely, a recent study of potential pathogens isolated from livestock and then inoculated onto spinach and lettuce in field plots reported rapid die-off of a shigatoxin-negative strain of *E. coli* O157:H7; this was in contrast to the survival of a strain of *S.* Enteritidis for at least 14 days (Hutchison and others 2008). These contrasting results emphasize again the variability of pathogen survival in complex environments, and the dependence of results probably upon pathogen fitness, experimental design (field versus microcosm), and other factors (spatial, temporal, indigenous flora, disease, etc.), any of which might

Table 1.5. Selected studies of the fitness of *E. coli* O157, *E. coli* O157:H7, and *Salmonella* in environmental samples or microcosms

Pathogen	Environment	Maximum Survival (Days)	Reference
E. coli O157:H7	Water, 8 °C	>91	Wang and Doyle 1998
	Water, 25 °C	<84	
E. coli O157	Water trough, sediment	245	LeJeune and others 2001
E. coli O157:H7	Water, <15 °C	14	McGee and others 2002
	Water + feces, <15 °C	24	
E. coli O157	Water, biofilms	>30	Cooper and others 2007
E. coli O157:H7	Water: lake, river, drinking trough microcosms	6 to >60 Lake > river	Avery and others 2008
E. coli O157	Soil	105	Ogden and others 2002
E. coli O157	Soil, manure-amended (child illness)	69	Mukherjee and others 2006
E. coli O157:H7	Soil, manure-amended	>35	Williams and others 2007
E. coli O157:H7 (Stx-neg)	Soil, 36 types	54–105	Franz and others 2008
E. coli O157:H7	Soil, cover crops	40–96	Gagliardi and Karns 2002
E. coli O157:H7	Manure, cow	47	Kudva and others 1998
	Manure, sheep	>600	
E. coli O157 (Stx-neg)	Feces, cow	97	Scott and others 2006
	Water	109	
E. coli O157:H7	Feces, cow, turned	42	Fremaux and others 2007
	Feces, cow, unturned	90	
E. coli O157:H7	Manure, cow	21	Himathongkham and others 1999
	Manure, slurry	35	
E. coli O157:H7 (Stx-neg)	Soil, manure-amended	154–217	Islam and others 2004, 2005
	Lettuce	77	
	Parsley	177	
	Onions	74	
	Carrots	168	
E. coli O157:H7 (Stx-neg)	Lettuce and spinach	<7	Hutchison and others 2008
S. enterica	Water, river	>45	Santo Domingo and others 2000
S. enterica	Soil, chicken farm	240	Davies and Breslin 2003
S. enterica	Soil	>120	Holley and others 2006
S. Newport	Soil, manure-amended	107–332	You and others 2006
	Manure, cow	49–184	
S. Enteritidis	Soil, almond orchard	>1500	Uesugi and others 2007
S. enterica	Soil, tomato crop debris (microcosm)[a]	56	Barak and Liang 2008
S. Enteritidis	Lettuce and spinach	>14 to <21	Hutchison and others 2008

[a] Some soils included crop debris from tomato plants infected with the pathogen *Xanthomanas campestris* and colonized with *S. enterica*.

in some combination be conducive to pathogen survival, growth, and, in some instances, increased virulence in a leafy vegetable–associated outbreak (Table 1.1). These results reflect a "snapshot" of the pathogen under the selected test or environmental conditions, in addition to a spectrum of fitness characteristics of the pathogen assessed.

Two studies relevant to concepts of persistence of specific pathogen strains in a preharvest environment and direct links to human illness are worth noting. A survey of a family garden subsequent to the O157:H7 illness of a child playing in the raw manure–amended garden revealed that strains indistinguishable from the child's strain were detectable in soil samples from the garden for >69 days, and that incidence was much higher in soil sampled during ambient temperatures compared to 4 °C (Mukherjee and others 2006). Similarly, strains of *S.* Enteritidis Phage Type 30 associated with at least one outbreak linked to raw almonds, and possibly a second (Table 1.1), were isolated over at least a 5-year period from soil drag swab samples obtained in an orchard linked to the outbreak (Uesugi and others 2007). The *Salmonella* strain, indistinguishable from outbreak strains, was isolated from soil more frequently during and after harvests (average 20–42% of samples, Aug–Dec), and in >50% of soil samples following a heavy rain event. Although the virulence and infectiousness of an environmental pathogen strain cannot be compared to related human clinical strains, the sets of *E. coli* O157:H7 and *S.* Enteritidis PT 30 environmental strains noted above are closely related epidemiologically to the corresponding clinical strains. It can be speculated that persistence of these pathogen strains in the garden and orchard environments may relate directly to the evolution of fitness characteristics that correlate also with virulence (Manning and others 2008).

Manure-amended soil, plants and plant debris appear to be beneficial to the survival of *E. coli* O157:H7 and *Salmonella* (Table 1.5). Ruminant-digested grasses and feeds and crop debris have nutrients supporting survival and possibly growth of enteric pathogens under the appropriate environmental conditions, including temperature, moisture, and atmosphere (Brandl 2006). For example, *E. coli* cells present naturally in cow feces placed in shaded and nonshaded fields increased 1.5 log after 6 to 8 days, declining fast in nonshaded fecal samples and then rebounding >1 log in nonshaded samples after rain events (Van Kessel and others 2007). In contrast, *E. coli* in air-dried sandy and silty soils amended with municipal sludge (biosolids) declined more slowly than in moist soils; up to 3 log differences were noted after 35 compared to 91 days in the field (Lang and Smith 2007). These studies are monitoring generic rather than pathogenic *E. coli*; however, the results are informative about different feces (cow, human), exposure to sun (UV) or moisture, and rates of resuscitation in rain—important environmental factors affecting pathogens in the environment. *E. coli* O157 and *S. enterica*, and generic *E. coli* as fecal indicator bacteria, appear capable of surviving months or even years under the appropriate environmental conditions and, under optimal conditions, they grow 1 to 3 logs (Table 1.5). Indeed, in a recent study of *Salmonella* in tomato crop debris, it appears this may be another aspect of the preharvest environment worth considering as a site conducive to survival or growth of pathogen for extended periods of time (Barak and Liang 2008). Tomato seeds planted in soil with *Salmonella*-contaminated tomato crop debris resulted in plants contaminated with *Salmonella* in the rhizoplane > phyllosphere. *Salmonella* survived

well in the tomato phyllosphere of plants from seeds inoculated with the tomato plant pathogen, *Xanthomonas campestris* pv. vesicatoria and planted in low *Salmonella* inoculum soil, indicating the potential importance of debris, plant disease, and fallow periods in the preharvest produce production cycle (Barak and Liang 2008). Thus, breakdown of tomato crop debris by plant pathogens may enhance the conditions for even better survival or growth of a human pathogen (Barak and Liang 2008; Brandl 2006; Brandl and Amundson 2008). Pathogen reservoirs where tenfold or more growth of pathogen may occur are critical risk factors relevant to food contamination. High-shedding animals; manure; crop and/or ground cover debris; and produce plant seedlings, leaves, and roots are candidate sites for amplification. Unidentified reservoirs of amplification, such as wild animals, microorganisms, and plants, may exist also.

Source-Tracking Pathogens and Fecal Indicators of Contamination in Watersheds

The epidemiology of major produce-associated outbreaks occurring in the last decade has revealed that preharvest contamination occurs (Table 1.1). However, surveys of fresh produce at different stages in the production and processing cycle indicate that bacterial pathogens are at low incidence generally (Beuchat 1996; Harris and others 2003; Nguyen-the and Carlin 1994, 2000), even though fecal indicator bacteria (*E. coli*) present appear to increase in prevalence during transport and distribution (Table 1.2) to wholesale and retail markets (Valentin-Bon and others 2008). Therefore, specific events following preharvest contamination are important to identify also since they may provide clues to amplification sites resulting in a high incidence or concentration.

An important stage in preharvest contamination is movement onto fields, and more importantly, onto or into seedlings or the mature plants. Water (Table 1.4; irrigation, flooding), intrusion by animals either directly (Table 1.3; wildlife, domestic, humans) or indirectly (fertilizer, compost), and dust are potential mechanisms of contamination. Water quality is a primary factor in production of safe fresh produce, and irrigation water comes from a variety of sources dependent upon the type of produce and location.

The majority of leafy vegetable production in the region of the U.S. implicated in outbreaks involves irrigation with well water of high quality relative to surface water that may be nearby. Indeed, well water was reported to be the source of irrigation of leafy vegetables associated with recent outbreaks (CalFERT 2007b, 2008). It is noteworthy also that U.S. winter produce production occurs mainly in the Imperial Valley of California and the Yuma region of Arizona, where irrigation water is sourced often from surface water. In contrast, outbreaks associated with produce from these locations have not occurred or have been rare (Table 1.1). Obviously, the quality of water in lakes, ponds, reservoirs, and watersheds is critical to produce production even when it is not used directly for irrigation. Surface water could be a major source of pathogens affecting aquifer recharging, exposure of animals to colonization, and/or transport to produce fields by irrigation, or processes as yet unidentified.

Watersheds are impaired by the presence of fecal bacteria from livestock, wildlife, and humans. Any fecal contamination increases the probability of enteric pathogen contamination of produce either directly or indirectly. The level of impairment is

dependent upon many factors related to the geography and ecology within and surrounding the watershed, including the density of animals, hydrology, elevation/runoff, meteorological conditions (e.g., rainfall and temperature), pathogen fitness (Table 1.5), water composition (salinity, nutrients), predation, and vegetation. Waterborne disease outbreaks in the U.S. (1948–1994) and Canada (1975–2001) occur more frequently following heavy rain events, indicating transport of pathogens from human, domestic animal, livestock, or wildlife sources through runoff, and, ultimately, contamination of drinking water supplies (Curriero and others 2001; Thomas and others 2006). Although no definitive links between heavy rain events and human illness have been reported, flood contamination of fields or irrigation water sources intended for growing produce is a potential risk factor for illness (CDHS 2005).

Watershed hydrology may be crucial to understanding pathogen transport within an environment. Hydrological processes are relevant to transport of pathogens in the environment, including fecal disintegration and dispersion, resuscitation of pathogens in arid environments, trapping of pathogens in wetlands, concentration of pathogens on or in sediment particles, land-to-watershed-to-land movement, and exposure of wildlife to pathogens (Ferguson and others 2003). Similarly, the soil and sediment particles present in flowing or static water bodies can interact and bind with microorganisms by mechanisms that are not well defined, and likely vary depending upon variations in soil, fecal and water composition, weather, and other factors (Gagliardi and Karns 2000; Brookes and others 2004; Ferguson and others 2003). Transport of pathogens in dust, on harvest equipment, in manure/compost and pesticide and herbicide sprays diluted with surface water should be considered also.

Pathogens and microbial species as indicators of fecal contamination can be prevalent in environments near produce production (Tables 1.3 and 1.4). Sensitive and accurate detection of specific pathogens in the environment to track the fate and transport of pathogens to fields requires intensive sampling, successful isolation of pathogens or fecal indicator microorganisms, and efficient molecular genotyping methods for microbial source tracking pathogens in relevant and complex environments (Field and Samadpour 2007; Meays and others 2004). A variety of different source tracking methods have been developed to identify sources of fecal contamination, sometimes yielding mixed results and accuracy (Field and Samadpour 2007; Stoeckel and others 2004). Microbial source tracking methods have evolved to include modern genetic methods that involve fingerprinting isolates from the environment and different animal hosts to create a database for comparing fingerprints of new strains to those in the database and thus identify putative sources of fecal contamination (Field and Samadpour 2007).

Pulsed field gel electrophoresis (PFGE) remains a common method for fingerprinting foodborne pathogens, mainly because of CDC's PulseNet database, which stores PFGE profiles submitted by public health labs representing tens of thousands of sporadic and outbreak strains for comparison (Swaminathan and others 2001). However, sequence-based typing methods, such as MultiLocus Variable number tandem repeat Analysis (MLVA), MultiLocus Sequence Typing (MLST), and Single Nucleotide Polymorphism (SNP) microarrays, are gaining in acceptance due to ease of use, speed, and high-resolution data for comparisons.

MLVA is an effective method for genotyping *E. coli* O157:H7 (Hyytia-Trees and others 2006) and is being evaluated also for *S.* Enteritidis. MLVA proved effective in

environmental studies involving tracking *E. coli* O157:H7 strains in produce production environments, watersheds, and cattle feedlots (Cooley and others 2007; Murphy and others 2008). An intriguing finding in the 2006 investigation of the *E. coli* O157:H7 multistate outbreak linked to bagged baby spinach was the isolation of multiple strains of *E. coli* O157:H7 from the feces of multiple feral swine trapped in the vicinity of the suspected spinach field; some of these isolates, and isolates from cow fecal, river. and dirt samples also collected within a mile of the field, were indistinguishable from the clinical outbreak strains (Jay and others 2007; Cooley and others 2007). Similarly, evidence of transport of *E. coli* O157:H7 strains between dairy farms by wild birds has been reported (Wetzel and LeJeune 2006).

How Do Pathogens Get onto Preharvest Produce and Survive?

Hypotheses from Recent Outbreaks

The transient incidence of pathogens in livestock, wildlife (Table 1.3), and watersheds (Table 1.4), the environmental fitness characteristics of foodborne pathogens (Table 1.5), and recurring outbreaks of foodborne illness associated with ready-to-eat produce (Table 1.1) are consistent with the findings of low-level, but significant, incidence of generic *E. coli* on fresh produce obtained from distribution centers and retail markets (Table 1.2). Although some of this *E. coli* could be present at harvest, postharvest contamination also could occur in a variety of ways, such as rodents, contaminated bins or transport vehicles, commingling of food at retail markets or restaurants, or ill workers. Postharvest cross-contamination could exacerbate what might have been a limited contamination event initially.

Preharvest contamination of produce occurs by obvious processes, but perhaps also by unknown, or less well understood, processes. Although no definitive conclusions have been offered about the sources of preharvest contamination of leafy vegetables and tomatoes associated with recent outbreaks (Table 1.1), reasonable hypotheses involve transport of pathogen in animal fecal waste by 1) watershed to flooded fields (CDHS 2005), 2) feral swine intrusion (Jay and others 2007), 3) irrigation by pipes used previously to remove dairy holding pond waste (CalFERT 2008), and 4) amphibian or other wild animals emerging from contaminated surface water to intrude into fields (MMWR 2005a).

Water is a central factor in hypotheses of contamination, so studies of the dispersion and dissemination of microbes in water and the use of microbes as tracers of water movement are relevant to understanding dissemination of enteric pathogens in water. Heavy rainfall is associated with rapid dispersal of pathogens from fecal matter on the ground into surface and groundwater (Ferguson and others 2003). Pathogen incidence and survival in feces, water, soil, and other matrices (Table 1.3, 1.4, 1.5) are relevant for modeling environmental contamination of preharvest produce, identifying sources, and controlling contamination, but details are lacking about how different species of bacteria, including pathogens, disperse and survive in water and other sites in the production environment and how this might relate to preharvest contamination.

Bacteria, yeasts, and bacteriophage have been used as tracers by dosing a large number of laboratory-grown cells (approximately 10^{14} cells) into a river and monitoring movement (Wimpenny and others 1972). The bacterial strain traced, *S. marcescens*

(distinctive red colonies), for example, moved in the river at approximately 2.5 km/hr over the 2.9 km between the dosing and detection points. The dosed strain was detected at a maximum of 500 cells/ml, which reflected a significant dilution (>1.7×10^8-fold) of the bacteria during transport (Wimpenny and others 1972). To achieve a comparable amount of *E. coli* O157:H7 from "high-shedder" cattle feces (e.g., 10^6 cells/g), for example, would require >200,000 kg of feces.

In a separate study in an elevated region within miles of leafy vegetable production, transport of *E. coli* O157:H7 strains was tracked from a point source (small corral with a few head of cattle) into a small stream (Cooley and others 2007). Indistinguishable or related pathogen strains identified by MLVA genotyping were isolated at the point source and up to 135 m downstream (3 m lower altitude) from the point source. However, water flow was relatively low prior to and at the time of sampling (Cooley and others 2007).

Isolation and/or detection of pathogens in water at distant sites from a suspected point source, therefore, might involve one or more of the following: large volumes of feces and/or high-shedding animals, very sensitive detection of few pathogen cells, multiple point sources with related strains, or transport mechanisms (e.g., cell-cell or cell-particulate aggregates, mats, flotation) different than those reflected by laboratory cultured microorganisms in tracer studies. Accurate tracer studies of pathogens in the environment would be advantageous for understanding fate and transport mechanisms relevant to produce contamination.

Pathogens in animal feces deposited on rangeland, feedlots, or dairy alleys, and into storage ponds are exposed to dispersion, transport, and inactivation that could be affected by soil and fecal matrices, particle sizes, buoyancy, microbial competitors/predators or cooperators, and even climate (rainfall, temperature, UV exposure). It is noteworthy that during the 2006 outbreak of *E. coli* O157:H7 associated with bagged baby spinach, unusually high daily temperatures occurred at the time of planting: July 22–25, 2006: max. daily 100–110 °F (37.7–43.3 °C); ave. daily 77–85 °F (25–29.4 °C), and approximately 5–6 days prior to harvest (CalFERT 2007b,c). This unusual condition stimulates questions regarding when contamination occurred in the crop cycle and whether high temperatures may have enhanced survival or growth of pathogen in the preharvest environment. For example, *E. coli* O157 has been shown to survive and increase in number with increasing temperature (10–30 °C) in natural freshwater microcosms containing low concentrations of organic carbon (Vital and others 2008). The direct correlation between pathogen growth and water temperature is consistent with enteric bacteria that have evolved to grow optimally at body temperatures.

Survival of Human Pathogens on Preharvest Plants

Outbreaks associated with preharvest contaminated produce confirm that enteric bacteria are capable of attaching somewhere on the plants and remaining viable (Tables 1.1 and 1.3). Field studies with nonpathogenic varieties of *E. coli* O157:H7 and other pathogens on plants under field conditions confirm that they can survive for weeks and months depending upon the amount of bacteria applied and the treatment conditions (Tables 1.2 and 1.3). Laboratory studies indicate that *E. coli* O157:H7 and *Salmonella* applied to a variety of plant roots, leaves, and seeds can attach tenaciously (resisting sanitization) and survive, but also in some instances grow when conditions

are ideal for a pathogen (warm temperature, high humidity, adequate nutrients) (Brandl 2006). Sophisticated fluorescence microscopy experiments have revealed specific locations on leaves and roots where subcuticlar cells, root hairs, or breaks in the tissue (e.g., lateral root formation) provide sites and nutrients for harboring opportunistic pathogen cells. Aggregation of enteric pathogen cells with one another and with plant epiphytic or plant pathogen microflora suggest that active and complex interactions may occur on plants in the field, resulting possibly in interactions/contamination very difficult to remove by normal washing or sanitizing methods (Brandl 2006). In addition, there appears to be emerging support for the hypothesis that some human pathogen cells on plants may become internalized through different routes of entry on roots, shoots, and flowers (Guo and others 2001; Solomon and others 2002; Warriner and others 2003; Dong and others 2003; Franz and others 2007; Doyle and Erickson 2008; Schikora and others 2008). Indeed, recent reports examining the plant response to potential human pathogens in model plant systems (*Arabidopsis thaliana* mutants and gene expression arrays) indicate that genes and gene pathways are upregulated similarly to plant resistance responses to plant pathogens (Dong and others 2003; Thilmony and others 2006; Schikora and others 2008). Thus, the potential for some human pathogens to be endopathogenic for some plant hosts in a preharvest environment raises obvious concerns regarding postharvest treatments for decontamination.

Reviews of different mechanisms that plant epiphytes and pathogens and human enteric pathogens use to attach to plants (Mandrell and others 2006; Solomon and others 2006) and an excellent review of the general biology, ecology, and fitness characteristics of human enteric pathogens on plants have been published previously (Brandl 2006). Further details about the molecular interactions that can occur between bacterial human pathogens (e.g., flagellin, fimbriae, pili, curli, outer membrane proteins) and plants (generally undefined), and the microbial ecology on plants that may enhance or control pathogen survival are provided in these reviews and also chapters elsewhere in this book.

Conclusions

The increased incidence of produce-related outbreaks tracked to specific regions, and *E. coli* O157:H7 outbreaks in particular, has stimulated questions about what might have changed over the last decade to explain this increase. Is it related to growing (fertilization, water, shallow tilling, seeds, cultivars) or production practices (cutting, transport, bagging, atmosphere), changes in the pathogens (increased fitness in animals, water), livestock (transport, incidence of pathogens), or better detection (methods, public health system, media)? Clearly, some of these questions raise issues that would be considered higher risk factors than others and worthy of prioritizing for research.

Most people can appreciate that animals or feces on or near fresh produce fields are major potential risk factors, probably worthy of attempts to prevent continued intrusion. Lacking convincing evidence of pathogen carriage by a suspect animal species, however, becomes problematic for making informed decisions about mitigation approaches (predation, fencing, testing). Indeed, lack of definitive proof of sources of pathogens has created a significant conflict between conservationists, environmentalists, and growers on one side versus those in the produce industry responsible for

addressing preharvest produce food safety issues. The conflict involves a contradiction between creation of vegetative zones for filtering runoff from fields and wildlife habitat, and the perceived risks of attracting to this habitat wildlife colonized possibly with pathogens (Berreti and Stuart 2008). Some compromise between these competing interests will be necessary for sustaining the valuable locations where produce is grown and improving the quality and safety of produce.

As noted above, a convergence of multiple events probably is required to cause a major outbreak, implying that each event alone may be insufficient. The changes in pathogen incidence and virulence in a preharvest food production environment can be speculated to be associated with corresponding and dynamic changes in the biology, ecology, hydrology, meteorology, and agricultural practices in an environment. Considering the impossibility of controlling certain aspects of the ready-to-eat produce production environment, it is logical to assume that additional outbreaks will occur. Intensive practices leading to exposure of pathogens to complex environments, or significant replication of microorganisms, will increase the rates of new mutations and fitness in environments where mutations are beneficial. Modern molecular biology techniques (genomics) are facilitating the fingerprinting of outbreak-related pathogen strains for purposes of high-resolution tracking of the possible sources of contamination in preharvest environments. Also, comparative genomics of these data reveal insights about pathogen evolution and emergence of virulence-related factors that raise questions about whether produce outbreak-related pathogens are more virulent and have special fitness characteristics (Zhang and others 2006; Manning and others 2008). The rapid changes possible in bacterial genomes by mutations, phage insertions and deletions, and recombination, as examples, predict the emergence from high-intensity environments (food production) of organisms with selected fitness characteristics that reflect the environment. If some of these fitness characteristics are virulence traits in humans (i.e., pathogens), pathogens will be identified through studies of human illness.

Considering the known potential risk factors in the preharvest environment documented above, some approaches for preventing contamination of food can be offered. Common sense approaches include maintaining water quality and minimizing exposure of fields to wild animals, surface water (flooding), and dust from agricultural activity. Other less obvious approaches requiring more resources are identifying high-shedding livestock or wildlife, treatment of livestock with effective vaccines or other antimicrobials, checking and maintaining feed quality, observing field conditions (wildlife intrusions), redirecting or destroying suspect produce, and controlling wild animal habitat. Postharvest approaches involve sample testing (test and hold), clean water, novel sanitizers (chemical or biological), and irradiation, to name a few. More details regarding interventions will be discussed in other sections of this book.

Finally, it should be noted again that the incidence of illness linked to contaminated produce is quite low relative to the total number of produce consumptions. Nevertheless, the increased incidence of outbreaks and the apparent hypervirulence of pathogen strains associated with some of these outbreaks (Manning and others 2008), emphasize that continued vigilance is necessary to minimize the severity of any outbreaks that might occur. Until a highly effective and nontoxic "kill step" is developed for eliminating pathogens from postharvest fresh produce, pathogens in the preharvest environment deserve our serious attention and continuing research efforts.

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References

Abong'o BO, Momba MNB and Mwambakana JN. 2008. Prevalence and antimicrobial susceptibility of *Escherichia coli* O157:H7 in vegetables sold in the Amathole District, Eastern Cape Province of South Africa. J Food Prot 71(4):816–819.

Ackers ML, Mahon BE, Leahy E, Goode B, Damrow T, Hayes PS, Bibb WF, Rice DH, Barrett TJ, Hutwagner L, Griffin PM and Slutsker L. 1998. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. J Infect Dis 177(6):1588–1593.

Arthur L, Jones S, Fabri M and Odumeru J. 2007a. Microbial survey of selected Ontario-grown fresh fruits and vegetables. J Food Prot 70(12):2864–2867.

Arthur TM, Bosilevac JM, Nou X, Shackelford SD, Wheeler TL and Koohmaraie M. 2007b. Comparison of the molecular genotypes of *Escherichia coli* O157:H7 from the hides of beef cattle in different regions of North America. J Food Prot 70(7):1622–1626.

Avery LM, Williams AP, Killham K and Jones DL. 2008. Survival of *Escherichia coli* O157:H7 in waters from lakes, rivers, puddles and animal-drinking troughs. Sci Total Environ 389(2–3):378–385.

Bailey JS, Stern NJ, Fedorka-Cray P, Craven SE, Cox NA, Cosby DE, Ladely S and Musgrove MT. 2001. Sources and movement of *Salmonella* through integrated poultry operations: a multistate epidemiological investigation. J Food Prot 64(11):1690–1697.

Barak JD and Liang AS. 2008. Role of soil, crop debris, and a plant pathogen in *Salmonella enterica* contamination of tomato plants. PLoS ONE 3(2):e1657.

Barkocy-Gallagher GA, Arthur TM, Rivera-Betancourt M, Nou X, Shackelford SD, Wheeler TL and Koohmaraie M. 2003. Seasonal prevalence of Shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. J Food Prot 66(11):1978–1986.

Baudart J, Lemarchand K, Brisabois A and Lebaron P. 2000. Diversity of *Salmonella* strains isolated from the aquatic environment as determined by serotyping and amplification of the ribosomal DNA spacer regions. Appl Environ Microbiol 66(4):1544–1552.

Berreti M and Stuart D. 2008. Food safety and environmental quality impose conflicting demands on Central Coast growers. Calif Agric 62:68–73.

Beuchat LR. 1996. Pathogenic microorganisms associated with fresh produce. J Food Prot 59(2):204–216.

———. 2006. Vectors and conditions for preharvest contamination of fruits and vegetables with pathogens capable of causing enteric diseases. Brit Food J 108(1):38–53.

Brandl MT. 2006. Fitness of human enteric pathogens on plants and implications for food safety. Annu Rev Phytopathol 44:367–392.

Brandl MT and Amundson R. 2008. Leaf age as a risk factor in the contamination of lettuce with *Escherichia coli* O157:H7 and *Salmonella enterica*. Appl Environ Microbiol 74(8):2298–2306.

Brandl MT, Haxo AF, Bates AH and Mandrell RE. 2004. Comparison of survival of *Campylobacter jejuni* in the phyllosphere with that in the rhizosphere of spinach and radish plants. Appl Environ Microbiol 70(2):1182–1189.

Branham LA, Carr MA, Scott CB and Callaway TR. 2005. *E. coli* O157 and *Salmonella* spp. in white-tailed deer and livestock. Curr Issues Intest Microbiol 6(2):25–29.

Breuer T, Benkel DH, Shapiro RL, Hall WN, Winnett MM, Linn MJ, Neimann J, Barrett TJ, Dietrich S, Downes FP, Toney DM, Pearson JL, Rolka H, Slutsker L and Griffin PM. 2001. A multistate outbreak of *Escherichia coli* O157:H7 infections linked to alfalfa sprouts grown from contaminated seeds. Emerg Infect Dis 7(6):977–982.

Brookes JD, Antenucci J, Hipsey M, Burch MD, Ashbolt NJ and Ferguson C. 2004. Fate and transport of pathogens in lakes and reservoirs. Environ Int 30(5):741–759.

CalFERT. 2007a. Environmental investigation of *Escherichia coli* O157: H7 outbreak associated with Taco Bell restaurants in Northeastern United States. California Food Emergency Response Team. Sacramento, CA. http://www.dhs.ca.gov/fdb/local/PDF/Taco_Bell_final_report_redacted_11_19_2007.pdf.

———. 2007b. Investigation of an *Escherichia coli* O157:H7 outbreak associated with Dole pre-packaged spinach. California Food Emergency Response Team. Sacramento, CA. http://www.dhs.ca.gov/ps/fdb/HTML/Food/EnvInvRpt.htm.

———. 2007c. Investigation of an *Escherichia coli* O157:H7 outbreak associated with Dole pre-packaged spinach. Attachment 10: Environmental samples from farms and watersheds. California Food Emergency Response Team. Sacramento, CA. http://www.dhs.ca.gov/ps/fdb/HTML/Food/EnvInvRpt.htm.

———. 2008. Investigation of the Taco John's *Escherichia coli* O157:H7 outbreak associated with iceberg lettuce. California Food Emergency Response Team. Sacramento, CA. http://www.dhs.ca.gov/fdb/HTML/Food/EnvInvRpt.htm.

Campbell JV, Mohle-Boetani J, Reporter R, Abbott S, Farrar J, Brandl M, Mandrell R, and Werner SB. 2001. An outbreak of *Salmonella* serotype Thompson associated with fresh cilantro. J Infect Dis 183(6):984–987.

Castillo A, Mercado I, Lucia LM, Martinez-Ruiz Y, Ponce de Leon J, Murano EA and Acuff GR. 2004. *Salmonella* contamination during production of cantaloupe: a binational study. J Food Prot 67(4):713–720.

CDC. Outbreak Surveillance Data, Annual Listing of Foodborne Disease Outbreaks, 1995 Foodborne Disease Outbreak Line Listing. January 4. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htm. Accessed February 25, 2008.

———. Outbreak Surveillance Data, Annual Listing of Foodborne Disease Outbreaks, 1996 Foodborne Disease Outbreak Line Listing. January 4. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htm. Accessed February 25, 2008.

———. Outbreak Surveillance Data, Annual Listing of Foodborne Disease Outbreaks, 1998 Foodborne Disease Outbreak Line Listing. January 4. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htm. Accessed February 25, 2008.

———. Outbreak Surveillance Data, Annual Listing of Foodborne Disease Outbreaks, 1999 Foodborne Disease Outbreak Line Listing. January 4. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htm. Accessed February 25, 2008.

———. Outbreak Surveillance Data, Annual Listing of Foodborne Disease Outbreaks, 2002 Foodborne Disease Outbreak Line Listing. January 4. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htm. Accessed February 25, 2008.

———. Outbreak Surveillance Data, Annual Listing of Foodborne Disease Outbreaks, 2003 Foodborne Disease Outbreak Line Listing. January 4. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htm. Accessed February 25, 2008.

———. Outbreak Surveillance Data, Annual Listing of Foodborne Disease Outbreaks, 2004 Foodborne Disease Outbreak Line Listing. January 4. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htm. Accessed February 25, 2008.

———. 2008a. Investigation of outbreak of infections caused by *Salmonella* Litchfield. March 22. Centers for Disease Control and Prevention. http://www.cdc.gov/salmonella/litchfield/. Accessed.

———. 2008b. Investigation of outbreak of infections caused by *Salmonella* Saintpaul. June 2. Centers for Disease Control and Prevention. http://www.cdc.gov/salmonella/saintpaul/. Accessed June 4, 2008.

CDHS. 2004a. Investigation of *E. coli* O157:H7 Illnesses in San Diego and Orange Counties California Department of Health Services. Sacramento, CA. http://www.dhs.ca.gov/ps/fdb/HTML/Food/EnvInvRpt.htm.

———. 2004b. Investigation of *E. coli* O157:H7 Outbreak in San Mateo Retirement Facility. California Department of Health Services. Sacramento, CA. http://www.dhs.ca.gov/ps/fdb/HTML/Food/EnvInvRpt.htm.

———. 2005. Addendum Report to Investigation of Pre-washed Mixed Bagged Salad following an Outbreak of *Escherichia coli* O157:H7 in San Diego and Orange County. California Department of Health Services. Sacramento, CA. http://www.dhs.ca.gov/ps/fdb/HTML/Food/EnvInvRpt.htm.

CDR. 2006. Enteric illness in the United Kingdom, 2005. Health Protection Agency. London. http://www.hpa.org.uk/CDR/archives/2006/cdr1506.pdf.

Chapman PA, Siddons CA, Gerdan Malo AT and Harkin MA. 1997. A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. Epidemiol Infect 119(2):245–250.

Chase-Topping ME, McKendrick IJ, Pearce MC, MacDonald P, Matthews L, Halliday J, Allison L, Fenlon D, Low JC, Gunn G and Woolhouse ME. 2007. Risk factors for the presence of high-level shedders of *Escherichia coli* O157 on Scottish farms. J Clin Microbiol 45(5):1594–1603.

Cizek A, Alexa P, Literak I, Hamrik J, Novak P and Smola J. 1999. Shiga toxin-producing *Escherichia coli* O157 in feedlot cattle and Norwegian rats from a large-scale farm. Lett Appl Microbiol 28(6): 435–439.

Clemens R. 2004. The expanding U.S. market for fresh produce. Iowa Ag Rev 10:Winter. http://www.card.iastate.edu/iowa_ag_review/winter_04/article4.aspx.

Cobbold RN, Rice DH, Davis MA, Besser TE and Hancock DD. 2006. Long-term persistence of multi-drug-resistant *Salmonella enterica* serovar Newport in two dairy herds. J Am Vet Med Assoc 228(4):585–591.

Cooley M, Carychao D, Crawford-Miksza L, Jay MT, Myers C, Rose C, Keys C, Farrar J and Mandrell RE. 2007. Incidence and tracking of *Escherichia coli* O157:H7 in a major produce production region in California. PLoS ONE 2(11):e1159.

Cooper IR, Taylor HD and Hanlon GW. 2007. Virulence traits associated with verocytotoxigenic *Escherichia coli* O157 recovered from freshwater biofilms. J Appl Microbiol 102(5):1293–1299.

CSPI. 2007. Outbreak Alert: Closing the gaps in our federal food safety net. Center for Science in the Public Interest, Washington, D.C. http://www.cspinet.org/foodsafety/outbreak_alert.pdf. Accessed March 3, 2008.

———. 2008. Outbreak Alert! Database, 1990–2005. Center for Science in the Public Interest, Washington, D.C. http://www.cspinet.org/foodsafety/outbreak/pathogen.php. Accessed March 22, 2008.

Cummings K, Barrett E, Mohle-Boetani JC, Brooks JT, Farrar J, Hunt T, Fiore A, Komatsu K, Werner SB and Slutsker L. 2001. A multistate outbreak of *Salmonella enterica* serotype Baildon associated with domestic raw tomatoes. Emerg Infect Dis 7(6):1046–1048.

Curriero FC, Patz JA, Rose JB and Lele S. 2001. The association between extreme precipitation and water-borne disease outbreaks in the United States, 1948–1994. Am J Public Health 91(8):1194–1199.

Dargatz DA, Fedorka-Cray PJ, Ladely SR, Kopral CA, Ferris KE and Headrick ML. 2003. Prevalence and antimicrobial susceptibility of *Salmonella* spp. isolates from US cattle in feedlots in 1999 and 2000. J Appl Microbiol 95(4):753–761.

Davies RH and Breslin M. 2003. Persistence of Salmonella enteritidis phage type 4 in the environment and arthropod vectors on an empty free-range chicken farm. Environ Microbiol 5(2):79–84.

Doane CA, Pangloli P, Richards HA, Mount JR, Golden DA and Draughon FA. 2007. Occurrence of *Escherichia coli* O157:H7 in diverse farm environments. J Food Prot 70(1):6–10.

Dong Y, Iniguez AL, Triplett EW and Ahmer BMM. 2003. Kinetics and strain specificity of rhizosphere and endophytic colonization by enteric bacteria on seedlings of *Medicago sativa* and *Medicago truncatula*. Appl Environ Microbiol 69(3):1783–1790.

Doyle MP and Erickson MC. 2008. Summer meeting 2007—the problems with fresh produce: an overview. J Appl Microbiol 105:317–330.

Duffy G. 2003. Verocytoxigenic *Escherichia coli* in animal faeces, manures and slurries. J Appl Microbiol 94Suppl:94S–103S.

Dunn JR, Keen JE, Moreland D and Thompson RA. 2004. Prevalence of *Escherichia coli* O157:H7 in white-tailed deer from Louisiana. J Wildl Dis 40(2):361–365.

Durso LM, Reynolds K, Bauer N, Jr. and Keen JE. 2005. Shiga-toxigenic *Escherichia coli* O157:H7 infections among livestock exhibitors and visitors at a Texas County Fair. Vector Borne Zoonotic Dis 5(2):193–201.

Ejidokun OO, Walsh A, Barnett J, Hope Y, Ellis S, Sharp MW, Paiba GA, Logan M, Willshaw GA and Cheasty T. 2006. Human Vero cytotoxigenic *Escherichia coli* (VTEC) O157 infection linked to birds. Epidemiol Infect 134(2):421–423.

Elder RO, Keen JE, Siragusa GR, Barkocy-Gallagher GA, Koohmaraie M and Laegreid WW. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing [see comments]. Proc Natl Acad Sci U S A 97(7):2999–3003.

FDA-CFSAN. 2001a. FDA survey of imported fresh produce, FY 1999 field assignment. January 30, 2001. http://www.cfsan.fda.gov/~dms/prodsur6.html. Accessed Mar. 3, 2008, 2008.

———. 2001b. Survey of domestic fresh produce: interim results (July 31, 2001). July 31, 2001. http://vm.cfsan.fda.gov/~dms/prodsur9.html. Accessed Oct. 16, 2002, 2002.

Feder I, Wallace FM, Gray JT, Fratamico P, Fedorka-Cray PJ, Pearce RA, Call JE, Perrine R and Luchansky JB. 2003. Isolation of *Escherichia coli* O157:H7 from intact colon fecal samples of swine. Emerg Infect Dis 9(3):380–383.

Fegan N, Vanderlinde P, Higgs G and Desmarchelier P. 2004. Quantification and prevalence of Salmonella in beef cattle presenting at slaughter. J Appl Microbiol 97(5):892–898.

———. 2005. A study of the prevalence and enumeration of Salmonella enterica in cattle and on carcasses during processing. J Food Prot 68(6):1147–1153.

Fenlon DR. 1981. Seagulls (*Larus* spp.) as vectors of salmonellae: an investigation into the range of serotypes and numbers of salmonellae in gull faeces. J Hyg (Lond) 86(2):195–202.

Ferguson C, De Roda Husman AM, Altavilla N, Deere D and Ashbolt N. 2003. Fate and transport of surface water pathogens in watersheds. Crit Rev Environ Sci Technol 33(3):299–361.

Field KG and Samadpour M. 2007. Fecal source tracking, the indicator paradigm, and managing water quality. Wat Res 41(16):3517–3538.

Fischer JR, Zhao T, Doyle MP, Goldberg MR, Brown CA, Sewell CT, Kavanaugh DM and Bauman CD. 2001. Experimental and field studies of *Escherichia coli* O157:H7 in white-tailed deer. Appl Environ Microbiol 67(3):1218–1224.

Foley SL, Lynne AM and Nayak R. 2008. *Salmonella* challenges: prevalence in swine and poultry and potential pathogenicity of such isolates. J Anim Sci 86:E149–162.

Franz E, Semenov AV, Termorshuizen AJ, de Vos OJ, Bokhorst JG and van Bruggen AH. 2008. Manure-amended soil characteristics affecting the survival of *E. coli* O157:H7 in 36 Dutch soils. Environ Microbiol 10(2):313–327.

Franz E, Visser AA, Van Diepeningen AD, Klerks MM, Termorshuizen AJ and van Bruggen AH. 2007. Quantification of contamination of lettuce by GFP-expressing *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium. Food Microbiol 24(1):106–112.

Fremaux B, Delignette-Muller ML, Prigent-Combaret C, Gleizal A and Vernozy-Rozand C. 2007. Growth and survival of non-O157:H7 Shiga-toxin-producing *Escherichia coli* in cow manure. J Appl Microbiol 102(1):89–99.

Froder H, Martins CG, De Souza KL, Landgraf M, Franco BD and Destro MT. 2007. Minimally processed vegetable salads: microbial quality evaluation. J Food Prot 70(5):1277–1280.

Gagliardi JV and Karns JS. 2000. Leaching of *Escherichia coli* O157:H7 in diverse soils under various agricultural management practices. Appl Environ Microbiol 66(3):877–883.

———. 2002. Persistence of *Escherichia coli* O157:H7 in soil and on plant roots. Environ Microbiol 4(2):89–96.

Garcia A and Fox JG. 2003. The rabbit as a new reservoir host of enterohemorrhagic *Escherichia coli*. Emerg Infect Dis 9(12):1592–1597.

Gaynor K, Park SY, Kanenaka R, Colindres R, Mintz E, Ram PK, Kitsutani P, Nakata M, Wedel S, Boxrud D, Jennings D, Yoshida H, Tosaka N, He H, Ching-Lee M and Effler PV. 2009. International foodborne outbreak of *Shigella sonnei* infection in airline passengers. Epidemiol Infect 137:335–341.

Greene SK, Daly ER, Talbot EA, Demma LJ, Holzbauer S, Patel NJ, Hill TA, Walderhaug MO, Hoekstra RM, Lynch MF and Painter JA. 2008. Recurrent multistate outbreak of Salmonella Newport associated with tomatoes from contaminated fields, 2005. Epidemiol Infect 136(2):157–165.

Guo X, Chen J, Brackett RE and Beuchat LR. 2001. Survival of salmonellae on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. Appl Environ Microbiol 67(10):4760–4764.

Gupta SK, Nalluswami K, Snider C, Perch M, Balasegaram M, Burmeister D, Lockett J, Sandt C, Hoekstra RM and Montgomery S. 2007. Outbreak of *Salmonella* Braenderup infections associated with Roma tomatoes, northeastern United States, 2004: a useful method for subtyping exposures in field investigations. Epidemiol Infect 135(7):1165–1173.

Hancock DD, Besser TE, Rice DH, Ebel ED, Herriott DE and Carpenter LV. 1998. Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in the Northwestern USA. Prev Vet Med 35:11–19.

Harris LJ, Farber JN, Beuchat LR, Parish ME, Suslow TV, Garret EH and Busta FF. 2003. Outbreaks associated with fresh produce: Incidence, growth, and survival of pathogens in fresh and fresh-cut produce. Comp Rev Food Sci Food Safety. pp. 78–89.

Hedberg CW, Angulo FJ, White KE, Langkop CW, Schell WL, Stobierski MG, Schuchat A, Besser JM, Dietrich S, Helsel L, Griffin PM, McFarland JW and Osterholm MT. 1999. Outbreaks of salmonellosis

associated with eating uncooked tomatoes: implications for public health. The Investigation Team. Epidemiol Infect 122(3):385–393.

Herman KM, Ayers TL and Lynch M. 2008. Foodborne disease outbreaks associated with leafy greens, 1973–2006. International Conference on Emerging Infectious Diseases. Atlanta, GA: Center for Disease Control and Prevention. p. 81.

Hernandez J, Bonnedahl J, Waldenstrom J, Palmgren H and Olsen B. 2003. Salmonella in birds migrating through Sweden. Emerg Infect Dis 9(6):753–755.

Hidalgo-Vila J, Diaz-Paniagua C, de Frutos-Escobar C, Jimenez-Martinez C and Perez-Santigosa N. 2007. Salmonella in free living terrestrial and aquatic turtles. Vet Microbiol 119(2–4):311–315.

Higgins JA, Belt KT, Karns JS, Russell-Anelli J and Shelton DR. 2005. *tir*- and *stx*-positive *Escherichia coli* in stream waters in a metropolitan area. Appl Environ Microbiol 71(5):2511–2519.

Hilborn ED, Mermin JH, Mshar PA, Hadler JL, Voetsch A, Wojtkunski C, Swartz M, Mshar R, Lambert-Fair MA, Farrar JA, Glynn MK and Slutsker L. 1999. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. Arch Intern Med 159(15):1758–1764.

Himathongkham S, Bahari S, Riemann H and Cliver D. 1999. Survival of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in cow manure and cow manure slurry. FEMS Microbiol Lett 178(2):251–257.

Holley RA, Arrus KM, Ominski KH, Tenuta M and Blank G. 2006. *Salmonella* survival in manure-treated soils during simulated seasonal temperature exposure. J Environ Qual 35(4):1170–1180.

Hughes LA, Shopland S, Wigley P, Bradon H, Leatherbarrow AH, Williams NJ, Bennett M, de Pinna E, Lawson B, Cunningham AA and Chantrey J. 2008. Characterisation of *Salmonella* enterica serotype Typhimurium isolates from wild birds in northern England from 2005 to 2006. BMC Vet Res 4(1):4.

Hussein HS and Bollinger LM. 2005. Prevalence of Shiga toxin-producing *Escherichia coli* in beef cattle. J Food Prot 68(10):2224–2241.

Hutchison ML, Avery SM and Monaghan JM. 2008. The air-borne distribution of zoonotic agents from livestock waste spreading and microbiological risk to fresh produce from contaminated irrigation sources. J Appl Microbiol 105:848–857.

Hyytia-Trees E, Smole SC, Fields PA, Swaminathan B and Ribot EM. 2006. Second generation subtyping: a proposed PulseNet protocol for multiple-locus variable-number tandem repeat analysis of Shiga toxin-producing *Escherichia coli* O157 (STEC O157). Foodborne Pathog Dis 3(1):118–131.

Ihekweazu C, Barlow M, Roberts S, Christensen H, Guttridge B, Lewis D and Paynter S. 2006. Outbreak of *E. coli* O157 infection in the south west of the UK: risks from streams crossing seaside beaches. Euro Surveill 11(4):128–130.

Isaacs S, Aramini J, Ciebin B, Farrar JA, Ahmed R, Middleton D, Chandran AU, Harris LJ, Howes M, Chan E, Pichette AS, Campbell K, Gupta A, Lior LY, Pearce M, Clark C, Rodgers F, Jamieson F, Brophy I and Ellis A. 2005. An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella enteritidis*. J Food Prot 68(1):191–198.

Islam M, Doyle MP, Phatak SC, Millner P and Jiang X. 2004. Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. J Food Prot 67(7):1365–1370.

———. 2005. Survival of *Escherichia coli* O157:H7 in soil and on carrots and onions grown in fields treated with contaminated manure composts or irrigation water. Food Microbiol 22(1):63–70.

Jay MT, Cooley M, Carychao D, Wiscomb GW, Sweitzer RA, Crawford-Miksza L, Farrar JA, Lau DK, O'Connell J, Millington A, Asmundson RV, Atwill ER and Mandrell RE. 2007. *Escherichia coli* O157:H7 in feral swine near spinach fields and cattle, central California coast. Emerg Infect Dis 13(12):1908–1911.

Johnson JY, Thomas JE, Graham TA, Townshend I, Byrne J, Selinger LB and Gannon VP. 2003. Prevalence of *Escherichia coli* O157:H7 and *Salmonella* spp. in surface waters of southern Alberta and its relation to manure sources. Can J Microbiol 49(5):326–335.

Johnston LM, Jaykus LA, Moll D, Anciso J, Mora B and Moe CL. 2006a. A field study of the microbiological quality of fresh produce of domestic and Mexican origin. Int J Food Microbiol 112(2):83–95.

Johnston LM, Jaykus LA, Moll D, Martinez MC, Anciso J, Mora B and Moe CL. 2005. A field study of the microbiological quality of fresh produce. J Food Prot 68(9):1840–1847.

Johnston LM, Moe CL, Moll D and Jaykus L. 2006b. The epidemiology of produce-associated outbreaks of foodborne disease. In: James J, editor. Microbial hazard identification in fresh fruit and vegetables. Hoboken, NJ: John Wiley and Sons. pp. 37–72.

Keen JE, Durso LM and Meehan TP. 2007. Isolation of Salmonella enterica and Shiga-toxigenic *Escherichia coli* O157 from feces of animals in public contact areas of United States zoological parks. Appl Environ Microbiol 73(1):362–365.

Keen JE, Wittum TE, Dunn JR, Bono JL and Durso LM. 2006. Shiga-toxigenic *Escherichia coli* O157 in agricultural fair livestock, United States. Emerg Infect Dis 12(5):780–786.

Keene WE, Sazie E, Kok J, Rice DH, Hancock DD, Balan VK, Zhao T and Doyle MP. 1997. An outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. JAMA 277: 1229–1231.

Kinde H, Castellan DM, Kass PH, Ardans A, Cutler G, Breitmeyer RE, Bell DD, Ernst RA, Kerr DC, Little HE, Willoughby D, Riemann HP, Snowdon JA and Kuney DR. 2004. The occurrence and distribution of *Salmonella enteritidis* and other serovars on California egg laying premises: a comparison of two sampling methods and two culturing techniques. Avian Dis 48(3):590–594.

Kirk JH, Holmberg CA and Jeffrey JS. 2002. Prevalence of *Salmonella* spp in selected birds captured on California dairies. J Am Vet Med Assoc 220(3):359–362.

Kudva IT, Blanch K and Hovde CJ. 1998. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. Appl Environ Microbiol 64(9):3166–3174.

Lang NL and Smith SR. 2007. Influence of soil type, moisture content and biosolids application on the fate of *Escherichia coli* in agricultural soil under controlled laboratory conditions. J Appl Microbiol 103(6):2122–2131.

Lawley TD, Bouley DM, Hoy YE, Gerke C, Relman DA and Monack DM. 2008. Host transmission of *Salmonella enterica* serovar Typhimurium is controlled by virulence factors and indigenous intestinal microbiota. Infect Immun 76(1):403–416.

Leclercq A and Mahillon J. 2003. Farmed rabbits and ducks as vectors for VTEC O157:H7. Vet Rec 152(23):723–724.

Ledet Muller L, Hjertqvist M, Payne L, Pettersson H, Olsson A, Plym Forshell L and Andersson Y. 2007. Cluster of *Salmonella* Enteritidis in Sweden 2005–2006—suspected source: almonds. Euro Surveill 12(6):E9–10.

LeJeune JT, Besser TE and Hancock DD. 2001. Cattle water troughs as reservoirs of *Escherichia coli* O157. Appl Environ Microbiol 67(7):3053–3057.

LeJeune JT, Besser TE, Rice DH, Berg JL, Stilborn RP and Hancock DD. 2004. Longitudinal study of fecal shedding of *Escherichia coli* O157:H7 in feedlot cattle: predominance and persistence of specific clonal types despite massive cattle population turnover. Appl Environ Microbiol 70(1):377–384.

Low JC, McKendrick IJ, McKechnie C, Fenlon D, Naylor SW, Currie C, Smith DG, Allison L and Gally DL. 2005. Rectal carriage of enterohemorrhagic *Escherichia coli* O157 in slaughtered cattle. Appl Environ Microbiol 71(1):93–97.

Mahon BE, Ponka A, Hall WN, Komatsu K, Dietrich SE, Siitonen A, Cage G, Hayes PS, Lambert-Fair MA, Bean NH, Griffin PM and Slutsker L. 1997. An international outbreak of Salmonella infections caused by alfalfa sprouts grown from contaminated seeds. J Infect Dis 175(4):876–882.

Makino S, Kobori H, Asakura H, Watarai M, Shirahata T, Ikeda T, Takeshi K and Tsukamoto T. 2000. Detection and characterization of Shiga toxin-producing *Escherichia coli* from seagulls. Epidemiol Infect 125(1):55–61.

Mandrell RE and Brandl MT. 2004. *Campylobacter* species and fresh produce: outbreaks, incidence and biology. In: Beier RC, Pillai SD, Phillips TD and Ziprin RL, editors. Preharvest and Postharvest Food Safety: Contemporary Issues and Future Directions. Ames, IA: IFT Press and Blackwell Publishing. pp. 59–72.

Mandrell RE, Gorski L and Brandl MT. 2006. Attachment of microorganisms to fresh produce. In: Sapers GM, Gorny JR and Yousef AE, editors. Microbiology of Fruits and Vegetables. Boca Raton, FL: CRC, Taylor and Francis. pp. 33–74.

Manning SD, Motiwala AS, Springman AC, Qi W, Lacher DW, Ouellette LM, Mladonicky JM, Somsel P, Rudrik JT, Dietrich SE, Zhang W, Swaminathan B, Alland D and Whittam TS. 2008. Variation in virulence among clades of *Escherichia coli* O157:H7 associated with disease outbreaks. Proc Natl Acad Sci U S A.

Matthews L, Low JC, Gally DL, Pearce MC, Mellor DJ, Heesterbeek JA, Chase-Topping M, Naylor SW, Shaw DJ, Reid SW, Gunn GJ and Woolhouse ME. 2006. Heterogeneous shedding of *Escherichia coli* O157 in cattle and its implications for control. Proc Natl Acad Sci U S A 103(3):547–552.

McCrea BA, Tonooka KH, VanWorth C, Boggs CL, Atwill ER and Schrader JS. 2006. Prevalence of *Campylobacter* and *Salmonella* species on farm, after transport, and at processing in specialty market poultry. Poult Sci 85(1):136–143.

McGee P, Bolton DJ, Sheridan JJ, Earley B, Kelly G and Leonard N. 2002. Survival of *Escherichia coli* O157:H7 in farm water: its role as a vector in the transmission of the organism within herds. J Appl Microbiol 93(4):706–713.

MDPH. Health officials investigate *E. coli* O157:H7 cases related to Dole prepackaged lettuce mixes sold at Rainbow Foods. Sept. 28, 2006. http://www.health.state.mn.us/news/pressrel/ecoli093005.html. Accessed.

Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM and Tauxe RV. 1999. Food -related illness and death in the United States. Emerg Infect Dis 5(5):607–625.

Meays CL, Broersma K, Nordin R and Mazumder A. 2004. Source tracking fecal bacteria in water: A critical review of current methods. J Environ Manag 73(1):71–79.

Meinersmann RJ, Berrang ME, Jackson CR, Fedorka-Cray P, Ladely S, Little E, Frye JG and Mattsson B. 2008. *Salmonella*, *Campylobacter* and *Enterococcus* spp.: their antimicrobial resistance profiles and their spatial relationships in a synoptic study of the Upper Oconee River basin. Microb Ecol 55(3):444–452.

Michel P, Wilson JB, Martin SW, Clarke RC, McEwen SA and Gyles CL. 1999. Temporal and geographical distributions of reported cases of *Escherichia coli* O157:H7 infection in Ontario. Epidemiol Infect 122(2):193–200.

Michino H, Araki K, Minami S, Takaya S, Sakai N, Miyazaki M, Ono A and Yanagawa H. 1999. Massive outbreak of *Escherichia coli* O157:H7 infection in school children in Sakai City, Japan, associated with consumption of white radish sprouts. Am J Epidemiol 150(8):787–796.

Miller WG and Mandrell RE. 2006. *Campylobacter* in the food and water supply: prevalence, outbreaks, isolation, and detection. In: Ketley J and Konkel ME, editors. *Campylobacter jejuni*: New Perspectives in Molecular and Cellular Biology. Norfolk, UK: Horizon Scientific Press. pp. 101–163.

Milnes AS, Stewart I, Clifton-Hadley FA, Davies RH, Newell DG, Sayers AR, Cheasty T, Cassar C, Ridley A, Cook AJ, Evans SJ, Teale CJ, Smith RP, McNally A, Toszeghy M, Futter R, Kay A and Paiba GA. 2008. Intestinal carriage of verocytotoxigenic *Escherichia coli* O157, *Salmonella*, thermophilic *Campylobacter* and *Yersinia enterocolitica*, in cattle, sheep and pigs at slaughter in Great Britain during 2003. Epidemiol Infect 136:739–751.

MMWR. 2002. Multistate outbreaks of *Salmonella* serotype Poona infections associated with eating cantaloupe from Mexico—United States and Canada, 2000–2002. MMWR Morb Mortal Wkly Rep 51(46):1044–1047.

———. 2004. Outbreak of *Salmonella* serotype Enteritidis infections associated with raw almonds—United States and Canada, 2003–2004. MMWR Morb Mortal Wkly Rep 53(22):484–487.

———. 2005a. Outbreaks of *Salmonella* infections associated with eating Roma tomatoes—United States and Canada, 2004. MMWR Morb Mortal Wkly Rep 54(13):325–328.

———. 2005b. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 sites, United States, 2004. MMWR Morb Mortal Wkly Rep 54(14):352–356.

———. 2007a. Multistate outbreaks of Salmonella infections associated with raw tomatoes eaten in restaurants—United States, 2005–2006. MMWR Morb Mortal Wkly Rep 56(35):909–911.

———. 2007b. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2006. MMWR Morb Mortal Wkly Rep 56(14):336–339.

Mohle-Boetani JC, Farrar J, Bradley P, Barak JD, Miller M, Mandrell R, Mead P, Keene WE, Cummings K, Abbott S and Werner SB. 2009. *Salmonella* infections associated with mung bean sprouts: epidemiological and environmental investigations. Epidemiol Infect 137:357–366.

Mohle-Boetani JC, Reporter R, Werner SB, Abbott S, Farrar J, Waterman SH and Vugia DJ. 1999. An outbreak of *Salmonella* serogroup Saphra due to cantaloupes from Mexico. J Infect Dis 180(4):1361–1364.

Moore JE, Wilson TS, Wareing DR, Humphrey TJ and Murphy PG. 2002. Prevalence of thermophilic *Campylobacter* spp. in ready-to-eat foods and raw poultry in Northern Ireland. J Food Prot 65(8):1326–1328.

Morabito S, Dell'Omo G, Agrimi U, Schmidt H, Karch H, Cheasty T and Caprioli A. 2001. Detection and characterization of Shiga toxin-producing *Escherichia coli* in feral pigeons. Vet Microbiol 82(3):275–283.

Mukherjee A, Cho S, Scheftel J, Jawahir S, Smith K and Diez-Gonzalez F. 2006. Soil survival of *Escherichia coli* O157:H7 acquired by a child from garden soil recently fertilized with cattle manure. J Appl Microbiol 101(2):429–436.

Mukherjee A, Speh D, Dyck E and Diez-Gonzalez F. 2004. Preharvest evaluation of coliforms, *Escherichia coli*, *Salmonella*, and *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers. J Food Prot 67(5):894–900.

Murphy M, Minihan D, Buckley JF, O'Mahony M, Whyte P and Fanning S. 2008. Multiple-locus variable number of tandem repeat analysis (MLVA) of Irish verocytotoxigenic *Escherichia coli* O157 from feedlot cattle: uncovering strain dissemination routes. BMC Vet Res 4:2.

Nakazawa M and Akiba M. 1999. Swine as a potential reservoir of shiga toxin-producing *Escherichia coli* O157:H7 in Japan. Emerg Infect Dis 5(6):833–834.

Nguyen-the C and Carlin F. 1994. The microbiology of minimally processed fresh fruits and vegetables. Crit Rev Food Sci Nutr 34(4):371–401.

———. 2000. Fresh and processed vegetables. In: Lund B, Baird-Parker T and Gould G, editors. The microbiological safety and quality of food. Gaithersburg, MD: Aspen. pp. 620–684.

Nielsen BB, Clausen B and Elvestad K. 1981. The incidence of *Salmonella* bacteria in wild-living animals from Denmark and in imported animals. Nord Vet Med 33(9–11):427–433.

Nuorti JP, Niskanen T, Hallanvuo S, Mikkola J, Kela E, Hatakka M, Fredriksson-Ahomaa M, Lyytikainen O, Siitonen A, Korkeala H and Ruutu P. 2004. A widespread outbreak of *Yersinia pseudotuberculosis* O:3 infection from iceberg lettuce. J Infect Dis 189(5):766–774.

Nygard K, Lassen J, Vold L, Andersson Y, Fisher I, Lofdahl S, Threlfall J, Luzzi I, Peters T, Hampton M, Torpdahl M, Kapperud G and Aavitsland P. 2008. Outbreak of *Salmonella* Thompson infections linked to imported rucola lettuce. Foodborne Pathog Dis. In press: Online.

Ogden ID, Hepburn NF, MacRae M, Strachan NJ, Fenlon DR, Rusbridge SM and Pennington TH. 2002. Long-term survival of *Escherichia coli* O157 on pasture following an outbreak associated with sheep at a scout camp. Lett Appl Microbiol 34(2):100–104.

Ogden ID, MacRae M and Strachan NJ. 2005. Concentration and prevalence of *Escherichia coli* O157 in sheep faeces at pasture in Scotland. J Appl Microbiol 98(3):646–651.

Oporto B, Esteban JI, Aduriz G, Juste RA and Hurtado A. 2008. *Escherichia coli* O157:H7 and non-O157 shiga toxin-producing *E. coli* in healthy cattle, sheep and swine herds in Northern Spain. Zoonoses Pub Health 55(2):73–81.

Pangloli P, Dje Y, Ahmed O, Doane CA, Oliver SP and Draughon FA. 2008. Seasonal incidence and molecular characterization of *Salmonella* from dairy cows, calves, and farm environment. Foodborne Pathog Dis 5(1):87–96.

Pritchard GC, Williamson S, Carson T, Bailey JR, Warner L, Willshaw G and Cheasty T. 2001. Wild rabbits—a novel vector for verocytotoxigenic *Escherichia coli* O157. Vet Rec 149(18):567.

Proctor ME, Hamacher M, Tortorello ML, Archer JR and Davis JP. 2001. Multistate outbreak of *Salmonella* serovar Muenchen infections associated with alfalfa sprouts grown from seeds pretreated with calcium hypochlorite. J Clin Microbiol 39(10):3461–3465.

Rasschaert G, Houf K, Van Hende J and De Zutter L. 2007. Investigation of the concurrent colonization with *Campylobacter* and *Salmonella* in poultry flocks and assessment of the sampling site for status determination at slaughter. Vet Microbiol 123(1–3):104–109.

Reller ME, Nelson JM, Molbak K, Ackman DM, Schoonmaker-Bopp DJ, Root TP and Mintz ED. 2006. A large, multiple-restaurant outbreak of infection with *Shigella flexneri* serotype 2a traced to tomatoes. Clin Infect Dis 42(2):163–169.

Renter DG, Gnad DP, Sargeant JM and Hygnstrom SE. 2006. Prevalence and serovars of *Salmonella* in the feces of free-ranging white-tailed deer (*Odocoileus virginianus*) in Nebraska. J Wildl Dis 42(3):699–703.

Renter DG, Sargeant JM, Hygnstorm SE, Hoffman JD and Gillespie JR. 2001. *Escherichia coli* O157:H7 in free-ranging deer in Nebraska. J Wildl Dis 37(4):755–760.

Rice DH, McMenamin KM, Pritchett LC, Hancock DD and Besser TE. 1999. Genetic subtyping of *Escherichia coli* O157 isolates from 41 Pacific Northwest USA cattle farms. Epidemiol Infect 122(3):479–484.

Rimhanen-Finne R, Niskanen T, Hallanvuo S, Makary P, Haukka K, Pajunen S, Siitonen A, Ristolainen R, Poyry H, Ollgren J and Kuusi M. 2009. *Yersinia pseudotuberculosis* causing a large outbreak associated with carrots in Finland, 2006. Epidemiol Infect 137:342–347.

Rose BE, Hill WE, Umholtz R, Ransom GM and James WO. 2002. Testing for *Salmonella* in raw meat and poultry products collected at federally inspected establishments in the United States, 1998 through 2000. J Food Prot 65(6):937–947.

Sagoo SK, Little CL and Mitchell RT. 2001. The microbiological examination of ready-to-eat organic vegetables from retail establishments in the United Kingdom. Lett App Microbiol 33(6):434–439.

Sagoo SK, Little CL, Ward L, Gillespie IA and Mitchell RT. 2003. Microbiological study of ready-to-eat salad vegetables from retail establishments uncovers a national outbreak of salmonellosis. J Food Prot 66(3):403–409.

Santo Domingo JW, Harmon S and Bennett J. 2000. Survival of *Salmonella* species in river water. Curr Microbiol 40(6):409–417.

Sargeant JM, Hafer DJ, Gillespie JR, Oberst RD and Flood SJ. 1999. Prevalence of *Escherichia coli* O157:H7 in white-tailed deer sharing rangeland with cattle. J Am Vet Med Assoc 215(6):792–794.

Schikora A, Carreri A, Charpentier E and Hirt H. 2008. The dark side of the salad: *Salmonella typhimurium* overcomes the innate immune response of *Arabidopsis thaliana* and shows an endopathogenic lifestyle. PLoS ONE 3(5):e2279.

Scott L, McGee P, Sheridan JJ, Earley B and Leonard N. 2006. A comparison of the survival in feces and water of *Escherichia coli* O157:H7 grown under laboratory conditions or obtained from cattle feces. J Food Prot 69(1):6–11.

Sewell AM and Farber JM. 2001. Foodborne outbreaks in Canada linked to produce. J Food Prot 64(11):1863–1877.

Seymour IJ and Appleton H. 2001. Foodborne viruses and fresh produce. J Appl Microbiol 91(5): 759–773.

Sivapalasingam S, Barrett E, Kimura A, Van Duyne S, De Witt W, Ying M, Frisch A, Phan Q, Gould E, Shillam P, Reddy V, Cooper T, Hoekstra M, Higgins C, Sanders JP, Tauxe RV and Slutsker L. 2003. A multistate outbreak of *Salmonella enterica* serotype Newport infection linked to mango consumption: impact of water-dip disinfestation technology. Clin Infect Dis 37(12):1585–1590.

Sivapalasingam S, Friedman CR, Cohen L and Tauxe RV. 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J Food Prot 67(10):2342–2353.

Soderstrom A, Osterberg P, Lindqvist A, Jonsson B, Lindberg A, Blide Ulander S, Welinder-Olsson C, Lofdahl S, Kaijser B, De Jong B, Kuhlmann-Berenzon S, Boqvist S, Eriksson E, Szanto E, Andersson S, Allestam G, Hedenstrom I, Ledet Muller L and Andersson Y. 2008. A large *Escherichia coli* O157 outbreak in Sweden associated with locally produced lettuce. Foodborne Pathog Dis 5(3):339–349.

Solomon EB, Brandl MT and Mandrell RE. 2006. Biology of foodborne pathogens on produce. In: Matthews KR, editor. Emerging Issues in Food Safety: Microbiology of Fresh Produce. Washington, D.C.: ASM Press. pp. 55–83.

Solomon EB, Yaron S and Matthews KR. 2002. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. Appl Environ Microbiol 68(1):397–400.

Soriano JM, Rico H, Molto JC and Manes J. 2001. *Listeria* species in raw and ready-to-eat foods from restaurants. J Food Prot 64(4):551–553.

Sproston EL, Macrae M, Ogden ID, Wilson MJ and Strachan NJ. 2006. Slugs: potential novel vectors of *Escherichia coli* O157. Appl Environ Microbiol 72(1):144–149.

Srikantiah P. 2002. Outbreak of *Salmonella* Javiana infections among participants of the 2002 US Transplant Games—Orlando, Florida, June 2002. Centers for Disease Control and Prevention. Atlanta, GA.

Stoeckel DM, Mathes MV, Hyer KE, Hagedorn C, Kator H, Lukasik J, O'Brien TL, Fenger TW, Samadpour M, Strickler KM and Wiggins BA. 2004. Comparison of seven protocols to identify fecal contamination sources using *Escherichia coli*. Environ Sci Technol 38(22):6109–6117.

Strachan NJ, Fenlon DR and Ogden ID. 2001. Modelling the vector pathway and infection of humans in an environmental outbreak of *Escherichia coli* O157. FEMS Microbiol Lett 203(1):69–73.

Swaminathan B, Barrett TJ, Hunter SB and Tauxe RV. 2001. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerg Infect Dis 7(3):382–389.

Tablante NL and Lane VM. 1989. Wild mice as potential reservoirs of *Salmonella* dublin in a closed dairy herd. Can Vet J 30(7):590–592.

Teunis P, Takumi K and Shinagawa K. 2004. Dose response for infection by *Escherichia coli* O157:H7 from outbreak data. Risk Anal 24(2):401–407.

Thilmony R, Underwood W and He SY. 2006. Genome-wide transcriptional analysis of the *Arabidopsis thaliana* interaction with the plant pathogen *Pseudomonas syringae* pv. tomato DC3000 and the human pathogen *Escherichia coli O157:H7*. Plant J 46(1):34–53.

Thomas KM, Charron DF, Waltner-Toews D, Schuster C, Maarouf AR and Holt JD. 2006. A role of high impact weather events in waterborne disease outbreaks in Canada, 1975–2001. Int J Environ Health Res 16(3):167–180.

Thunberg RL, Tran TT, Bennett RW, Matthews RN and Belay N. 2002. Microbial evaluation of selected fresh produce obtained at retail markets. J Food Prot 65(4):677–682.

Tuyet DT, Yassibanda S, Nguyen Thi PL, Koyenede MR, Gouali M, Bekondi C, Mazzi J and Germani Y. 2006. Enteropathogenic *Escherichia coli* O157 in Bangui and N'Goila, Central African Republic: A brief report. Am J Trop Med Hyg 75(3):513–515.

Uesugi AR, Danyluk MD, Mandrell RE and Harris LJ. 2007. Isolation of *Salmonella* Enteritidis phage type 30 from a single almond orchard over a 5-year period. J Food Prot 70(8):1784–1789.

USDA-AMS-MDP. U.S. Department of Agriculture, Agricultural Marketing Service, Microbial Data Program. Data summaries, 2002–2006. July 9, 2008, Washington, DC. http://www.ams.usda.gov/science/mpo/Mdp.htm. Accessed July 23, 2008.

USDA-ERS. 2001. Lettuce: In & Out of the Bag. Washington, DC. http://www.ers.usda.gov/publications/AgOutlook/April2001/AO280d.pdf.

Valentin-Bon I, Jacobson A, Monday SR and Feng PC. 2008. Microbiological quality of bagged cut spinach and lettuce mixes. Appl Environ Microbiol 74(4):1240–1242.

Van Kessel JS, Pachepsky YA, Shelton DR and Karns JS. 2007. Survival of *Escherichia coli* in cowpats in pasture and in laboratory conditions. J Appl Microbiol 103(4):1122–1127.

Vital M, Hammes F and Egli T. 2008. *Escherichia coli* O157 can grow in natural freshwater at low carbon concentrations. Environ Microbiol 10:2387–2396.

Wahlstrom H, Tysen E, Olsson Engvall E, Brandstrom B, Eriksson E, Morner T and Vagsholm I. 2003. Survey of *Campylobacter* species, VTEC O157 and *Salmonella* species in Swedish wildlife. Vet Rec 153(3):74–80.

Wang G and Doyle MP. 1998. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in water. J Food Prot 61(6):662–667.

Warriner K, Ibrahim F, Dickinson M, Wright C and Waites WM. 2003. Internalization of human pathogens within growing salad vegetables. Biotechnol Genet Eng Rev 20:117–134.

WDOH. *E. coli* infections detected in two Puget Sound counties. June 5, 2008. Health WSDo, Seattle, WA. http://www.doh.wa.gov/Publicat/2008_news/08-092.htm. Accessed June 12, 2008.

Wetzel AN and LeJeune JT. 2006. Clonal dissemination of *Escherichia coli* O157:H7 subtypes among dairy farms in northeast Ohio. Appl Environ Microbiol 72(4):2621–2626.

Williams AP, Avery LM, Killham K and Jones DL. 2007. Survival of *Escherichia coli* O157:H7 in the rhizosphere of maize grown in waste-amended soil. J Appl Microbiol 102(2):319–326.

Wimpenny JWT, Cotton N and Statham M. 1972. Microbes as tracers of water movement. Wat Res 6:731–773.

You Y, Rankin SC, Aceto HW, Benson CE, Toth JD and Dou Z. 2006. Survival of *Salmonella enterica* serovar Newport in manure and manure-amended soils. Appl Environ Microbiol 72(9):5777–5783.

Zhang W, Qi W, Albert TJ, Motiwala AS, Alland D, Hyytia-Trees EK, Ribot EM, Fields PI, Whittam TS and Swaminathan B. 2006. Probing genomic diversity and evolution of *Escherichia coli* O157 by single nucleotide polymorphisms. Genome Res 16(6):757–767.